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CLINICAL SHOCK; A STUDY OF THE BIOCHEMICAL RESPONSE TO INJURY IN MAN.

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ABSTRACT

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At the University of Maryland School of Medicine a Clinical Shock Unit (CSU) has been established to study the biochemical response to injury in man.

Traumatic shock is associated usually with severe injury and characterized principally by inability to maintain an adequate circulation. This study focuses on the total problem - the reaction of the body to injury, maintenance of life, and repair of injury.

Studies currently in progress and those proposed are aimed primarily to understanding the biochemical response to injury in man. Provisions have been made for careful metabolic studies in the shocked patient without interfering with obvious life saving measures.

Such extensive studies has required the assembly of a considerable staff - professional and technical to support a C.S.U. on a 24 hour basis.

ABSTRACT

Experimental problems relevant to establishment of such a unit evolved from two major factors. (1). Original nature of the study (a scientific study of shock in man) (2). An unprecedented design of this study. Solutions to these problems are described.

Since inception of the contract January, 1962, some 200 patients have been studied as they have undergone recussitation measures. Final organization of the unit now permits more complex studies into the physio-biochemical response to injury in man.

7. Key Words:

Shock	Shock Therapy
Hemorrhagic Shock	Hypothermia
Septic Shock	Hyperbaric Oxygen
Cardiogenic Shock	Aldosterone
Strangulation Obstruction	Balanced Electrolyte Solutions
Ammonia Metabolism	Mitochondria Infusion
Modified Fine Technique	Hyperbaric Oxygenation
Irreversibility	Hypoxia
Shock Mechanisms	Clinical Shock Unit

CONTENTS

I. PREFACE

Since the last Progress report covering period of January 1, 1962 to March 20, 1963 minor modifications in the protocol have become necessary but the basic philosophy and objectives have remained the same.

Essentially, we know that shock is usually associated with severe injury and is characterized principally by an impaired circulation. Because of this striking and readily discernable situation, most studies of shock have been directed primarily at circulatory factors, their cause and correction. In the shock state, the biochemical integrity of the cell determines to a large extent whether or not the patient will survive. The shock syndrome, as such, obviously is a sum total of many interrelated and yet independent factors and mechanisms. If this condition is to be prevented or treated adequately, each factor or mechanism must be analyzed. Considerable information has been gained through extensive animal studies. These experiences are being put to work in our clinical study according to the protocol submitted by this contract.

Since the fundamental theme of our proposal is the study of shock at the cellular level in the human, our objectives are: (a) elucidation of the biochemical and physiologic alteration in shock in man; (b) development of therapeutic regimens; (c) development of preventive measures. The mechanics of operation are two-fold: (1) Clinical Shock Research Center to include special patient wards, laboratories, operating

rooms, specialized study and therapy areas (such as OHP chambers), and a data processing area; and (2) Animal Research Center for concomitant and correlative controlled investigations.

These objectives have first resulted in the Army pilot Clinical Shock Unit already described in the previous report. A future Shock Trauma Center will allow expansion of the above objectives and mechanisms of operation by the creation of a complete, self-contained, integrated shock study program.

For organization purposes in our programming, specific areas studied are sub-divided since the protocol is aimed primarily for investigation of cause and effect. Concomitant therapy as a life saving measure is an integral part of the study. Emphasis on new potential such as hyperbaric oxygenation (OHP) will be used whenever indicated. For organizational purposes in programming, the specific areas to be studied are as follows:

A. Study of the Mechanism in Shock

   1. Metabolism
   2. Physiology
      a. Cardio-respiratory
      b. Circulation
         1. Radio-isotope techniques
      c. Renal

B. Evaluation of OHP, hypothermia, and combination of the two, as therapeutic tools.

C. The role of immuno-bacteriological problems in shock.

D. Evaluation of anesthetic problems and agents in OHP.

E. Animal research to further augment the study and treatment of shock.

1. Enzyme Studies
2. Chemotherapy in Hemorrhagic Shock
3. Cardiogenic Shock

II. PROGRESS OF CLINICAL SHOCK UNIT (CSU) TO DATE

A. Organization

The organization of the Clinical Shock Unit, at the present time, consists of the Principal Investigator responsible to the Chairman of the Department of Surgery and he, in turn, is supported by the Director of the Clinical Unit and the Director of the supporting Animal Research Unit.

The Clinical Shock Unit employs two biochemists at the Ph.D. level and one at the M.D., Ph.D. level. Negotiations are being carried on with the University of Wisconsin Enzyme Research Laboratory for a third Ph.D. biochemist. The Unit has been fortunate in obtaining the services of Robert Ollodart, M.D. and Manfred Strauch, M.D. Doctor Ollodart, a surgeon with a Master degree in Bacteriology and Immunology has set up a laboratory to study the bacterial and immunological factors in shock. Doctor Strauch, a physician from the University of Heidelberg, with a background in pathology and renal physiology joined the Unit this summer after having spent 18 months in the University of Maryland Hypertension laboratory. Doctor Strauch is presently working in renal metabolism as it relates to the anoxic kidney.

Two new Laboratory Scientist IV positions were created to adequately supervise and train technical personnel, modify present technical methods, develop new methods and assist in the research planning. There are 14 technicians at various grade levels supporting the Unit on a 24 hour basis.

The shock ward, per se, employs six nurses, four of whom are supported by the contract and the remaining two by the University.

The patient area of the Shock Trauma Unit was originally planned for a patient capacity of four. The past eight months experience in caring for critically ill patients in the small four bed unit indicates that the space is not adequate to care for more than two patients at a time. Monitoring equipment, and the other necessary specialized equipment for study and therapy of the patients in shock occupy the space of two beds. Therefore, with the present facilities available, the Unit has reduced its bed capacity by two; making this unit a two-bed area. In addition, the type of patients admitted to the Unit during the first 24-48 hours, requires 16-24 nursing care hours per patient.

The surgical team, composed of attending surgical staff, is well organized and has been in operation since the opening of the Unit. In many instances, this has created hardships over the past year and it was apparent that Fellowship appointments were necessary. As of September 1, 1963, five such individuals were made available to the Unit on a twenty-four hour rotating basis. All but one Fellow is supported by University sources. The main responsibility of these physicians is to perform the necessary cannulations and assure that the protocol studies are met during the period of patient resuscitation. Although this plan has not been in effect for very long, it has demonstrated its value.

All members of the Clinical Shock Unit, including technical personnel, meet once a week, wherein problems encountered during the previous week are discussed and future plans made. One night meeting per month is devoted to a progress report submitted by one of the study areas.

One severe detriment to the program has been that of inadequate laboratory space. Dean William S. Stone has partially alleviated this situation by the allocation of the Third Floor of the Bressler Building to the Shock Unit (10,141 gross sq.ft.; 8,933 net sq.ft.). This additional space will relieve the overcrowded hospital area. Many laboratories are now being moved into this area. Other laboratories await renovation funds being sought through private sources.

B. Contributions

Dean William S. Stone has made available to the Clinical Shock Unit, funds amounting to approximately $65,000. Five thousand dollars for renovation of the enzyme laboratory; $32,000 to equipment; and $28,000 for the cinefluoroscopy unit used for the necessary complex catheterization studies required.

A 22' x 8' hyperbaric experimental chamber has been acquired from the Dixie Manufacturing Company. This $58,000 chamber was purchased through the USPHS Grant No. HE-07898-01 at a cost of $23,500, thus relieving the Army contract of that obligation. The remainder of the purchase price, $34,500, was donated by the Dixie Manufacturing Company. An additional $25,000 has been obtained through private sources to install

this unit in the Clinical Shock area on the third floor Bressler laboratory. Bids for installation of this facility on the third floor of the Bressler Building are underway.

The Hercules Powder Company, Wilmington, Delaware, has donated a hyperbaric chamber to the Shock Unit. This is a large clinical chamber, the main unit of which will be 13' x 25' and contain a lock 15' x 8'. This award includes the necessary engineering and equipment at an estimated cost of $250,000. The main chamber has been transported to the Dixie Manufacturing Company who will subcontract the completion of the unit. The University is in the process of acquiring a site, as well as the estimated $100,000 necessary to house and furnish the ancillary areas for this operation room facility. Fabrication of this chamber will be completed in four to six months.

A USPHS Grant No. RC-1151 has been awarded to the University of Maryland for the construction of a Shock Trauma Center. To assist in seeking private contributions for the additional $800,000 matching funds for this Center, a brochure was created. This brochure contains photographs and details of the Shock Trauma Unit at present, photographs of the Dixie Hyperbaric Chamber and Hercules Hyperbaric Chamber, and future plans for development of the Center.

An additional grant obtained to help support the Shock Trauma Center is USPHS Grant No. HE-07898-01 which will allow the purchase of a hyperbaric oxygen chamber. This grant will also staff this complex with an investigator, technician and engineer.

III. NEW STUDIES INITIATED

A. Microcirculation

The Shock Trauma Unit has been fortunate in obtaining the part time services of Doctor Melvin K. Knisely to establish and develop a microcirculation section of the Shock Unit. Doctor Knisely will train one of our technicians at his own expense at the Medical College of South Carolina. This technician on returning to the Shock Unit will carry out microcirculation studies during his periods of absence. It is hoped that additional funds will be available through the University to acquire Doctor Knisely on a full-time basis.

B. Clotting Mechanisms

Our preliminary studies in the clotting mechanisms of blood have shown that this phenomena may play an important role in the shock syndrome and will require more concentrated and elaborate investigation. A new laboratory is being set up for this purpose.

In addition to the hemogram, the determination of coagulation time, prothrombin time, and fibrinogin; platelet count will be added to these tests that will demonstrate the using up of all blood elements in intravascular clotting in shock. Better standardization of some of these tests, especially the coagulation and prothrombin times will be made whenever the coagulometer is obtained. The partial thromboplastin time test will also be determined with the coagulometer. A thromboelastograph has been ordered and will enable us to study graphically all phases of

clotting fibrin formation and fibrinolysis. Further studies of fibrinolysis will be determined by evaluation of fibrinolysis using the euglobin test and the determination of endogenous heparin. Further study of intravascular coagulation will be carried out in the determination of cryoprofibrin which was isolated by Shainoff and Page. The formation of cryoprofibrin is the only presently available chemical evidence for intravascular thrombin activity and is expected to increase its states of shock. Study of the proteolytic activity of the blood in states of shock will be undertaken; specifically the determination of bradykinnin and its possible relation to the plasma kallikrien system.

C. Medical Systems Analyses and Control

The Unit has been aware, since its inception, that baseline data gathered on shock patients has not been classified or analyzed. Furthermore, it is becoming apparent that more applied physics and engineering such as rheology, should be included. The amount of data in many areas is overwhelming and it became apparent that a method of data processing is necessary. Doctor William H. Kirby, an M.D. Engineer, with experience in medical systems analysis and control, has consented to be a consultant for the Unit in this field. Doctor Kirby performs a similar function for the Ballistic Research Laboratories, Aberdeen Proving Ground, advising and assisting biomedical engineering programs supporting the Army's wounding ballistics effort. To initiate this program, he will use a Ph. D. mathematician to assist in planning and

controlling the research effort. The objective of Doctor Kirby's studies will be to determine a feasible method for information handling and data analysis to meet the needs of the Clinical Shock Research Program. The scope of the study will cover three areas of activity which relate to complex research data handling and support:

1. Information storage, retrieval, and display methods.
2. Data analysis techniques including mathematical and statistical methods with possible on line considerations. System analysis including feedback ideas at the cellular level are suggested.
3. Analog, digital and hybrid analog-digital computing and information handling facilities considering both on-line and off-line needs.
4. Supportive applied physics and engineering.

The above methods should enable the unit to investigate areas which appear productive as well as terminate non-productive research activities. A curriculum vitae in reference to Doctor William H. Kirby, Jr., is enclosed.

A monitoring and recording system to handle such complex shock data is nearly constructed. This unit is designed to use the IBM computer recently located in the School of Medicine as well as the large IBM Computer Center at College Park. (See Appendix A)

D. Portal Vein Cannulation

A technique of portal vein cannulation via the umbilical vein is being taught to the Research Fellows. This method devised by Holbrook is essentially that of dissecting the umbilical vein and passing a catheter

into the portal system. This will be of inestimable value in metabolic studies concerning the liver.

E. OHP - Model for Normal Physiology

Hyperbaric oxygenation is playing a more prominent role in the shock program. Since three atmospheres has been employed in these laboratories, and elsewhere for management of potential clinical problems, it is necessary to establish this laboratory norm. This will entail a complete study of the physiologic, biochemical, and enzymatic changes under these conditions for periods of exposure of approximately two hours. The study is to emphasize cardio-vascular changes particularly regional distribution of flow and reflex mechanisms. The biochemical study is to emphasize acid-base metabolism, lactate, and pyruvate metabolism, and oxydative enzyme systems. These are to be done in the dog. It is also proposed to investigate the effect of OHP on the problem of oxidative phosphorylation. This is to be done by study of mitochondria changes in the heart and in the liver in the rat under these OHP conditions.

IV. SECTION PROGRESS REPORTS

A. STUDY OF THE MECHANISMS IN SHOCK

1. Metabolism

It is our assumption that the ultimate fate of the shock patient lies in the functioning state of the enzyme system. Thus, in order to get definite and possibly decisive information regarding the metabolic responses to shock, we are collecting a multitude of biochemical data in three different group arrangements:

1. Normal human individuals
2. Shock patients (hemorrhagic, septic, traumatic shock)
3. Animals (dogs, rabbits, rats)*

* These experiments are performed to study different stages of shock under controlled conditions and to check organs which are not accessible to identical studies in men.

TECHNIQUES — Outline of procedures

A. TISSUE

I. Study of Enzyme Systems

1) Measurement of single reaction steps
   a) in tissue homogenates
   b) in subcellular fractions
   c) isoenzyme determinations
   d) study of enzyme kinetics

2) Semiquantitative survey of metabolic pathways by means of combined techniques

II. Estimation of steady state levels of metabolites in tissue samples

B. BODY FLUIDS

I. Enzyme activities in blood plasma

II. Metabolite concentrations in blood

III. Metabolites in urine

All enzyme studies are restricted to living tissues taken by means of biopsy techniques. In clinical research samples of heart muscle (both atrium and ventricle), skeletal muscle, liver and kidney will be checked. In animal research the study is extended to brain, spleen and suprarenal glands. Heart biopsies are only practicable during open heart operations. They are performed to learn how the heart muscle reacts upon the stress of operation and to control the effects of artifical circulation, hypothermia and OHP. All the other tissues are easily accessible.

With an amount of 80-100 mg tissue (wet weight) up to 25 different enzymes can be checked per sample. Immediately following the biopsy the tissue is carefully blotted, cleaned, weighed and homogenized at low temperatures. The enzyme activities are determined in the supernatent obtained after ultracentrifugation of the homogenate.

Most of the enzymes selected are linked to main pathways of metabolism: glycolysis (EMBDEN-MEYERHOF-pathway) and oxidation (KREBS-cycle). Some belong to essential metabolic sidepaths (e. g. DICKENS-HORECKER pentose monophosphate shunt) or are closely connected with the urea cycle and the amino acid metabolism. Four different enzymes checked are specially involved in energy metabolism (kinases). In alphabetic order the selected enzymes are:

1. Adenosintriphosphatase (ATPase)
2. Aldolase (ALD)
3. Creatinephosphokinase (CPK)
4. Enolase (ENOL)
5. Glutamate Dehydrogenase (GLDH)
6. Glutamic-Oxaloacetate Transaminase (GOT)
7. Glutamic-Pyruvic Transaminase (GPT)
8. Glucose-6-phosphate Dehydrogenase (G-6-PDH)
9. Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH)
10. Glycerol-1-phosphate Dehydrogenase (GDH)
11. Isocitric Dehydrogenase (ICDH)

12. Lactic Dehydrogenase (LDH)
13. Malic Dehydrogenase (MDH)
14. Myokinase (Adenylatekinase (MK)
15. 6-Phosphogluconic Dehydrogenase (PGDH)
16. Phosphoglucose Isomerase (PGI)
17. Phosphoglycerate Kinase (PGK)
18. Phosphorylase a,b (PhL)
19. Pyruvic Kinase (PK)
20. Triosephosphate Isomerase (TIM)

With the exception of ATPase and Phosphorylase all these enzymes are determined spectrophotometrically using modern optic enzymatic tests (WARBURG). It is possible to determine the activity of all twenty enzymes in each of three to four different samples within one day (requires two technicians). The measured activity is expressed as units per gram wet weight, as well as in units per gram extracted protein.

Tissue samples with higher blood contents require the determination of the percentage of contamination for erythrocytes contain reasonable amounts of certain enzymes. This is done by comparison of the hemoglobin contents of the homogenate and peripheral blood. The enzyme activity found in the respective erythrocytes in a separate test is then subtracted from the tissue enzyme activity according to the calculated blood contents (mainly for G-6-PDH, MK and PGDH).

To prevent a false interpretation of the results regarding the measured tissue enzyme activities there must be a routine histological examination of each tissue section. All organ samples contain various amounts of connective tissue and fat which are additional sources of extractable enzymes (especially GLDH, G-6-PDH, and PGDH). Under pathological conditions these amounts and quantitative relations may change thus causing a distortion of the typical

enzyme pattern of the tissue in question.

Whenever possible election microscope examinations of the tissue samples will be carried out. Many enzymes are attached to subcellular structures for instance to mitochondria and microsomes. By means of this technique it should be possible to decide whether biochemical changes occur prior to structural alterations or not.

Under certain conditions one will have to compare tissue enzyme activities and histological state with simultaneously determined enzyme levels in the peripheral blood. In order to understand the observed changes and see the connections it is necessary to know the intracellular distribution of all the checked enzymes in all the organs in question.

Information about the intracellular distribution of enzymes in various human organs is as yet only partly available. This investigation requires greater amounts of tissue being subjected to differential centrifugation and using resected organs, parts thereof or autopsy material of patients very shortly after death (e.g. traffic accidents). A standard technique for this procedure has been worked out. The examination will yield a survey of the percentage distribution of each enzyme within the obtained four cell fractions: nuclei, mitochondria, microsomes and cytoplasm.

These cell components are well characterized from a morphologic point of view through their structural geometry, their dimensions and frequently through their internal organization. However, a detailed morphologic analysis by means of electron microscopy is necessary for all the biochemical work on the various cell fractions, to check the fractionation technique used,

and to correlate the isolated particles with structural elements observed in the intact cell.

Enzymes of identical catalytic function from various organs can be separated into different enzyme proteins of identical specifity. These heterogenous enzyme proteins are called "Isoenzymes" or "Isozymes". As mentioned later on (s.B,I) the determination of isoenzymes in blood plasma is of great diagnostic importance, indicating cellular damage in a certain organ. Our investigations will first include the electrophoretic separation of LDH and MDH isoenzymes in all the biopsy material obtained.

Simultaneously performed isoenzyme separations in tissue and serum will yield more information about several important points, such as the permeability of cell membranes to enzymes under both normal and pathological conditions, the velocity of permeation in relation to the subcellular enzyme distribution and the velocity, as well as, the manner of enzyme elimination from peripheral blood.

Since isoenzymes of different subcellular origin vary even in their properties (e.g. substrate affinity) these determinations should facilitate the localization of the very first site of action of a pathological process in question.

Tissue enzymes which show substantial alterations in their activities compared to normal conditions will be subjected to studies of their kinetics. These studies will include the determinations of MICHAELIS constants, the rate of inhibition by known inhibitors, the influence of pH, ions, temperature and various other analytical characterizations. The changes observed should substantiate the existing knowledge of the mode of enzyme inactivation or aging.

In addition to the determination of single reactions and enzymes in standard preparations some cases will require the application of various combined techniques to give over-all picture of metabolic pathways. Whenever possible we will first try to determine in vivo by means of isotopic tracer techniques what specific metabolic reactions occur in the tissues of the whole organism and then make further studies of these tissues in vitro to find out how these reactions are controlled.

The main interest is directed to the metabolic pathways of glycolysis - including the PASTEUR effect - of oxidative phosphorylation including the counter action of ATPase and to amino acid synthesis and - breakdown. For this purpose tissue slices as well as homogenates and isolated mitochondria will be checked, using combined manometric and spectrophotometric techniques. These methods are applied with the intention of studying the regulatory factors that determine which metabolic sequences occur when many alternatives are possible or which reactions are rate-controlling.

Special attention will be paid to the structure and biochemical function of mitochondria during shock. Localized in these particles is the normal cellular respiration, i.e. the oxidative metabolism and the terminal electron transport ("respiratory chain"). Thus, they play the most important role in energy metabolism, producing about 95% of the total energy necessary for life.

The measurement of mitochondrial oxidative phosphorylation and ATPase activity will be restricted almost exclusively to animal experiments, for the isolation of mitochondria in reasonable amounts requires more tissue than can be obtained by means of biopsy (of course, it will be possible in amputation

or resection preparations). However, recently developed techniques allow the assay of these functions and of enzymes and coenzymes of the respiratory chain in very small amounts of tissue (photoelectric spectrophotometry, using the "double beam spectrophotometer" of B. CHANCE). It will be possible to include these methods as soon as the necessary equipment is available.

The activity of tissue enzymes in vitro is not necessarily identical with their activity in vivo. Inhibitors present in the living cell and preventing full activity of certain enzymes in vivo may be removed during tissue preparation procedures leading to higher activities in vitro. The release of structural fixed enzymes from their cellular carriers - for instance from mitochondria by effective homogenization - will partly cause the same effect. Moreover, there is a great difference between the substrate and ion concentrations, the pH value and many other factors under the conditions in vivo and the artifical "physiological" conditions in vitro.

Therefore, in order to attain a more actual survey of the metabolic situation it is indispensable to determine simultaneously steady state levels of metabolites besides the mentioned enzymes.

Animal experiments have proved the insufficiency of most technical procedures used to prepare tissue samples for the analysis of metabolites: the conventional biopsy and following deproteinization of tissue samples require too much time allowing drastic changes in the concentration of these compounds. During biopsy - within milliseconds - most of the important metabolites, for instance, the adenosine and pyridine nucleotides will be

catabolized or converted to "operational isomers".

The most useful method to prevent these alterations is the application of the so-called "freeze-stop technique": a special forceps precooled to -190° C cuts off a piece of tissue, pressing it to a thin layer and cooling it down to -100°C within parts of a second. This step is followed by immediate deproteinization of the sample, performed in a cold room, and using liquid air to control the temperature down.

As yet, this technique has not been applied to human research. This is the main reason for the lack of satisfactory data concerning the metabolite contents of human organs under various conditions. Freeze-stop forceps developed for animal research are not applicable to human conditions. In co-operation with the Baltimore Instruments Co. the development of a special small forceps suitable to our planned investigations in humans is now in progress. The following metabolites will be determined:

1. Adenosine-mono-phosphate (AMP)
2. Adenosine-di-phosphate (ADP)
3. Adenosine-tri-phosphate (ATP)
4. Cretinephosphate (CP)
5. Dihydroxyacetonephosphate (DAP)
6. Fructose-6-phosphate (F-6-P)
7. Fructose-1, 6-diphosphate (FDP)
8. Glucose (Gluc)
9. Glucose-6-phosphate (G-6-P)
10. D-Glyceraldehyde-3-phosphate (GAP)
11. - Ketoglutarate (KG)
12. Lactic Acid (LA)
13. Nicotineamide-adenine-dinucleotide, oxidized and reduced form (NAD, NAD-H)
14. Nicotineamide-adenine-dinucleotide-phosphate, and reduced form (NADP, NADP-H)
15. Phospho-enol-pyruvate (PEP)
16. Pyruvic Acid (PA)

In addition inorganic phosphorus (Pi) determinations are necessary for turnover studies of these energy-rich phosphate compounds.

With the exception of inorganic phosphorus, the quantitative analysis of these compounds will be performed by means of the most modern enzymatic techniques. Methods and necessary reagents are on hand.

Moreover, the improved biopsy technique will enable us to determine the actual concentration of free amino acids in tissue. A special auto-analyzer is already in use for the assay of amino acids in serum. In connection with the other values obtained this data should give an excellent survey of the respective metabolic situation.

Already under physiological conditions a multitude of cell enzymes permeates the cell membranes or is even secreted from organ cells into the circulating blood. The majority of these enzymes belongs to the main pathways of the energy-generating metabolism that is, they can be found in every tissue of the body. Some enzymes, however, are localized almost exclusively in one organ; thus, any change of their activity in the plasma points specifically to alterations of this organ. These enzymes are called "organ-specific enzymes".

Generally, a pathological destruction of tissue cells increases the amount of tissue enzymes in blood serum. A choice of several enzymes determined simultaneously will therefore be of great diagnostic value.

The following serum enzyme activities will be checked in the course of our study; organs which are main sources of these enzymes are mentioned in parenthesis.

1. ALD (liver, kidney, skeletal muscle)
2. CPK (heart, skeletal muscle "organ-specific")
3. GLDH (liver mitochondria. "cell fraction specific")
4. GOT (heart, liver, kidney)
5. GPT (liver)
6. G-6-PDH (connective-, fat-and lymphatic tissue)
7. LDH (skeletal muscle, heart, kidney, liver, erythrocytes)
8. MDH (liver, heart, skeletal muscle, brain, erythrocytes)
9. SDH (liver "organ-specific")

* Sorbitol-Dehydrogenase

Additional measurements concern the "plasma specific" enzymes prothrombine, factors V and VII.
About 15 ml blood are needed to get the necessary amounts of serum for the assay of all enzymes.

Obviously, many organs of the body will be involved and more or less functionally disturbed during the course of shock. As preliminary studies have shown, this will increase the activity of most of these enzymes in the peripheral blood, thus deteriorating the value of serum enzyme determinations regarding organ specifity of diagnosis (not regarding the possibilities for the continuous observation of the course of the disease and of the prognosis). Therefore, it seems to be much more advantageous to analyze blood samples taken directly from blood vessels close to the organ in question rather than from the cubital vein. This requires different forms of catheterization. However, this technique is already in use for other clinical purposes and can be easily utilized for enzyme studies.

A better way to obtain an organ specific diagnosis without catheterization is the assay of isoenzymes in serum from peripheral blood. It is planned to separate the LDH and MDH isozymes in serum by means of a special electrophoresis. The trespass of organ-LDH or -MDH into the serum causes typical

alterations of the isozyme peaks in the serum electropherogram, thus indication the origin of any excess activity in serum. As mentioned previously (A, 1 c), isozyme determinations in blood and tissue samples are supposed to be of special importance for the investigation of certain biochemical responses to shock.

In agreement with the remarks made in #A, it is important to determine in addition to the enzyme activities also various metabolite concentrations in blood. Hereby, again the survey of the actual metabolic situation will be improved.

With the exception of CP, DAP, GAP, NAD-H, and NADP-H the same metabolites, as mentioned in #A,II for tissue, will be determined in blood; urea is assayed additionally. Here, too, the quantitative analysis will be performed with the aid of enzymatic techniques which have been used previously. About 15 ml blood are necessary for the estimation of all metabolites. It is essential that after drawing the blood it is immediately deproteinized in ice cold perchloric acid.

Some of the metabolites to be determined in whole blood are localized in the corpuscular elements of blood and do not occur free in the plasma (especially the adenosine and pyridine nucleotides, the hexose phosphates and the energy-rich phosphate compounds). Because plasma and erythrocyte volume will change during shock, it is always necessary to determine the hematocrit as a means of reference.

Using a routine micro-method, values for blood pH, standard bicarbonate and pCO_2 will also be available. In special cases plasma NH, will

be determined, too. The continuation of the assay of amino acid in serum by means of auto-analyzer techniques and lipid determinations by means of gas chromatography will complete the metabolic studies in blood.

Urine will be analyzed for urea to see whether the relation between blood urea and urine urea can give some information about the kidney function during shock.

SUMMARY OF PROGRESS IN THE ENZYME LABORATORY

The establishment of an Enzyme Laboratory, as part of the Shock Unit, was completed on May 3, 1963. Within the following two weeks, the equipment was installed and standardized.

Because of the introduction of new and difficult techniques, the one technician available since May 3rd, had to be trained over a period of five weeks. On June 6th, the first studies were begun. Below is an outline of the work completed so far.

1) Comparison of various methods for protein determinations was made using standardized protein solutions and Homogenates of red organs. The protein estimation by ultra-violet absorption (Warburg-Christian) and by Lowry using the Folin-Ciocalteu reagent seems most suitable for the enzyme work. It is, of course, necessary to check in further studies, the agreement of these methods with the results obtained by Kjeldahl technique. At this time we do not have the necessary equipment for the latter.

2) Using Homogenate of Rat Brain, the activity of the following enzymes has been determined: *GOT, GPT, LDH, and MDH. We are now

beginning to compare the values obtained under normal conditions, with those in Noble-Collip drum shock. This work is done in cooperation with Doctor Michaelis and Doctor Komatsu.

3) In a series of studies, the enzymatic response to anoxia in dog kidney is checked. The following enzymes are determined: *GAPDH, GOT, GPT, G-6-PDH, ICDH, LDH, MDH in:

a) Kidney homogenates before ligation of the renal artery

b) Kidney homogenates four hours after ligation

c) Kidney homogenates one to three weeks post-ligation

This work is done in cooperation with Dr. Strauch. The study up to this time has been on 15 dogs, and will be continued.

4) In tissue samples of human heart (atrium and ventricle) obtained during open-heart operations, the following 18 enzymes are determined: *ALD, CPK, ENOL, GAPDH, GDH, GLDH, GOT, GPT, G-6-PDH, ICDH, MDH, MK, PGDH, PGI, PGK, PK, TIM.

This study so far, has included 11 atrium samples, and 6 ventricle samples, taken before putting the patient on the pump oxygenator. This study will be continued including enzyme determinations on samples obtained during different phases of the operation.

5) A modified technique has been worked out for the electrophoretic separation of Isozymes using Cellulose Acetate Strips. This is being done using serum and various tissue homogenates. Satisfactory results have been obtained with the Isozymes of LDH, and MDH. The adaptation of this method to other enzymes is being tried.

1) ALD - Aldolase
2) CPK - Creatinephosphokinase
3) ENOL - Enolase
4) GLDH - Glutamate Dehydrogenase
5) GOT - Glutamic-Oxaloacetate Transaminase
6) GPT - Glutamic-Pyruvic Transaminase
7) G-6-PDH - Glucose-6-phosphate Dehydrogenase
8) GAPDH - Glyceraldehyde-3-phosphate Dehydrogenase
9) GDH - Glycerol-1-phosphate Dehydrogenase
10) ICDH - Isocitric Dehydrogenase
11) LDH - Lactic Dehydrogenase
12) MDH - Malic Dehydrogenase
13) MK - Myokinase (Adenylatekinase)
14) PGDH - 6-Phosphogluconic Dehydrogenase
15) PGI - Phosphoglucose Isomerase
16) PGK - Phosphoglycerate Kinase
17) PK - Pyruvic Kinase
18) TIM - Triosephosphate Isomerase

The purpose of this program is the exploration of the basic biochemical mechanisms in shock and their alteration during shock in man. The following basic biochemical studies are deemed important in our research program.

1. Hematocrit - micro-method

2. Hemoglobin - alkaline hematin colorimetric method

3. Refractive Index - by using Goldberg Refractometer, the specific gravity, total solid concentration, and total protein concentration are determined on serum and urine.

4. Blood pH, pCO_2, pO_2, CO_2, and O_2 content; and % saturation - Determined by Astrup Micro Technique, manometric, tonometer and electronic methods.

5. Electrolytes -

   A. Sodium and Potassium - flame photometer
   B. Magnesium - fluorometric determination
   C. Chlorides - titrimetric method

6. Chemistries -

   A. Urea Nitrogen: Karr's Method
   B. Glucose: Method of Folin - Wu
   C. Blood Ammonia: Seligson Method
   D. Lactate Acid: Barker - Summerson
   E. Pyruvic Acid: Freedman - Haugen

In the future, plans are being made to better standardize the chemical analyses already being performed. Also to add kidney function and clearances of creatinine, para amino hippuric acid and insulin. In order for this to be done, it is necessary to have a separate complete laboratory and technicians with more adequate training.

7. Amino Acids -

Amino acid determinations are being performed on blood plasma, urine and tissue taken from patients in shock. Blood samples taken during subliminal perfusion as well as a substantial number of normals have been studied. (See Appendix - B)

The samples are prepared and analyzed according to the methods outlined in the following papers: Stein, W.H., J. Biol. Chem 201, 45-48 (1953), Stein, W.H., Moore, S., J. Biol. Chem 211, 915-926 (1954), Tallan H.H., Moore, S., Stein, W.H., J. Biol. Chem 211, 927-939 (1954). At present, the preparation and complete analysis of a physiological sample on the Beckman 120-B Amino Acid Analyzer requires a total of 52 hours. However, with the addition of an Accelerated Run Conversion Kit to a present 120-B Amino Acid Analyzer in the near future, it will be possible to analyze three samples in the same 52 hour period.

This laboratory is being equipped for the separation and identification of peptides and related compounds from various biological materials. On completion we will be capable of quantitatively studying the blood for possible abnormal or increased amounts of polypeptides such as bradykinin wnich ma be released during increased proteolysis of plasma protein constituents in the shock syndrome.

7A. Electrophoretic plasma protein studies and amino acid analyses are being done on identical shock and normal samples in an effort to correlate amino acid variations with plasma protein levels in shock.

If it is found that certain plasma protein fractions are related to amino acid variations in shock, we will then attempt to isolate, purify and eventually characterize the proteolytic enzymes responsible

8. Gas Chromatography

The techniques for total steroids, fatty acids, aromatic amines and polysaccharide fractions are being established.

Numerous abnormalities in the serum lipid of patients in traumatic shock have been reported. We are already applying gash chromatography to determine from the sera of a group of shock patients (1) the total lipid, (2) total neutral lipid, (3) total phospholipid and (4) the fatty acid composition of the total lipid fraction, the cholesterol ester fraction, the triglyceride fraction and the phospholipid fraction.

At present, twenty sera samples from three shock patients and one normal control patient are under analysis using the modified micro-technique of Folch M. Lees for sera lipid analysis (J. Biol. Chem. Vol. 226, 1957). Analysis of the above data awaits re-evaluation of the present techniques used.

2. Physiology

The fluorography-isotope laboratory has not as yet become functional because of the critical space shortage. Overflow from the biochemical laboratory necessitated utilizing this area to begin the fundamental studies of metabolic changes in hypoxia. However, funds have been made available to move the enzyme laboratory to the third floor of the Bressler Building. This will allow exploration of certain organs under catheter-fluorography control for sampling.

Information is not available in reference to normal human cardiac tissue enzymes. Whenever possible biopsy of the atrium and ventricle prior to and following pump oxygenator perfusion will offer a means of collecting living cardiac tissue for enzyme sampling. The following enzymes are being determined at the present time: ALD, CPK, ENOL, GAPDH, GDH, GLDH, GOT, GPT, G-6-PDH, ICHD, MDH, MK, PGDH, PGI, PGK, PK, TIM.

This procedure will offer a norm for a comparative study of the cardiac enzyme activity during traumatic injury requiring thorocotomy for resuscitation. As the laboratory enzyme techniques and procedures improve, this same method will be used to study liver tissue, i.e., biopsy of the normal liver (when attainable) will be compared with shocked liver tissue obtained when laporatomy is used as a life-saving measure.

Coagulation Studies

A hypothesis of disseminate intravascular thrombosis has been proposed by Col. R. M. Hardaway to explain the various changes occurring in shock that ultimately lead to irreversibility. This hypothesis has been

supported by experimental studies that demonstrated a decrease in fibrinogen level, a prolongation in clotting, and prothrombus time and a decrease in the platelets in shock secondary to hemorrhage, endotoxins, injections of thrombus or mismatched blood. The validity of this hypothesis was tested in patients admitted to the shock unit in various stages of shock. Initial blood samples were withdrawn as soon as the patient was admitted to the unit. Repeated samples were obtained within 4 - 7 hours and then every 24 hours if the clinical status of the patient remains the same. If there is a change in the clinical condition or surgery is performed, blood samples were obtained before and after surgery, until the patient is discharged or expires.

The following tests were performed:

1) Fibrinogen level using the turbidimetric method of Parfentjer normal values 200 - 400 mgm%.

2) Clotting time - Lee White's method using silicone tubes. Normal values 20 - 60 min.

3) Prothrombin time Quick method normal values 14 - 17 seconds.

4) Hemoglobin and hematocrit using the micromethod.

From March 13, 1963 till August 2, 1963, the following tests were performed:

383 tests for Fibrinogen

264 tests for Clotting time

284 tests for Prothrombin time

Thirty-five patients are included in this study. The clinical diagnosis was

as follows:

5 Acute myocardial and pulmonary infarctions.

9 Septic shock

16 Hemorrhagic shock

3 Trauma

1 Shock cause (?) Terminal carcinoma

1 Burn

Changes in Fibrinogen:

A. Hemorrhagic shock

The most common cause of hemorrhagic shock studied in this group was bleeding duodenal ulcer. There were 3 cases secondary to rupture of major vessels (2 aorta), and 1 case of hematuria. In general, there was a correlation between the arterial blood pressure as a measure of shock and the fibrinogen level: whenever the mean arterial blood pressure was above 60 mm Hg (except in hypertensive patients). The fibrinogen level was either in the normal range or above. As the bleeding continued and the blood pressure dropped below a mean of 60 mm Hg, hypofibrinogenemia occurred to levels below 50 mgm% in some cases. This was a bad prognostic sign, for despite restoration of fibrinogen levels to normal values, these patients ultimately died. However, this general rule was not universal. There were patients in hemorrhagic shock from 5 hours to a terminal condition with mean blood pressures varying from 0 - 50 mm Hg., hematocrits 18-25% and hemoglobins 5 - 8 gms%, and yet exhibited normal fibrinogen levels. Seven patients fell into the latter group. Although this can be accounted for by the administration of blood in certain instances which tend to restore the fibrinogen level, yet

the majority had been in shock both initially and terminally, without exhibiting the picture of hypofibrinogenemia.

B. Septic Peritonitis

There were 9 cases of septic shock, mostly secondary to peritonitis. Five cases demonstrated hypofibrinogenemia, where 4 others had normal fibrinogen levels (average 33 mg), althought there was a slight decrease in the fibrinogen level. All patients in septic peritonitis died, irrespective of the fibrinogen levels of the blood, except one secondary to septic abortion who survived.

C. Acute Myocardial Infarction

There were 5 patients with acute myocardial and pulmonary infarctions, in cardiogenic shock which demonstrated a marked drop in the fibrinogen levels, with the onset of cardiogenic shock. (average 123 mg%)

Changes in Clotting Time:

A. Hemorrhagic Shock

In 6 patients the clotting time was normal and in 8 other patients it followed an abnormal pattern - a hypercoagulable state was observed immediately during the bleeding episode which was restored to normal state in patients that survived, or changed to a hypocoagulable state which was observed in terminal patients. The blood clotted in the syringe in some patients whereas in the terminal patient blood would not clot in more than 3 hours. The test was not

B. Septic Shock

Only 3 patients had normal clotting times, 3 patients had clotting times varying between 2 - 4 hours and 3 exhibited the hypercoagulable state initially (clotting time 7 - 16 minutes) followed by a state of hypocoagulability.

The only patient who survived (septic abortion) had an initial clotting time of 9' which became prolonged to 2 hours 24 hours later. However it returned to normal values a few days later.

C. Acute Myocardial Infarction

Two patients had a prolonged clotting time up to 4 hours, in contro-distinctions to 2 others who had an initial short clotting time of 6 minutes which became prolonged to 2 hours 25 minutes as the cardiogenic shock persisted.

Changes in Prothrombin Time:

A. Hemorrhagic Shock

Ten patients had normal prothrombin time. Six survived and 4 died. The remaining 6 had prolongation of the prothrombin time (an average of 33% normal). One patient whose prothrombin time increased from 16' to 35' (22% of normal) improved on treatment and his prothrombin time became normal, 14 seconds (100%).

B. Septic Shock

There were only 2 patients with normal prothrombin time, one of whom survived. The remaining patients demonstrated an average prothrombin time of 34% normal. The prolongation of the prothrombin time beyond 40% normal was a bad prognostic sign, since all these patients died.

C. Myocardial Infarction

A prolongation of the prothrombin time was uniformly seen in patients with myocardial infarctions ranging from 8 - 41% of normal.

In summary, the changes in fibrinogen, clotting and prothrombin

times produced by disseminated intravascular coagulation demonstrated in more than 50% of patients in irreversible hemorrhagic, septic and cardiogenic shock. These changes carried a bad prognostic significance. However they could not be demonstrated in a large percentage of shock patients, despite the severity of the shock state.

Occlusion Studies

The successful outcome of any study is in no small measure dependent upon experimental design. The controlled experimental study of shock in dogs is a relatively simple undertaking. This is especially true since resuscitation of the shocked animal is not necessarily critical. By contrast, the study of shock in humans is rightfully limited and modified by the ultimate therapeutic goal - the successful resuscitation of a human life. There are however, certain clinical situations which allow for controlled study of "reversible shock" in humans. It is the purpose of this paper to report one such "preparaton" and to present preliminary data which lends support to the thesis that "shock" of a reversible nature exists in a limited body segment and is amenable for controlled study.

The extremities distal to the cross clamped aorta would seem to offer an ideal situation for the study of reversible shock in humans. More specifically this "preparation" lends itself to the study of metabolic changes which might occur in the periphery under conditions of anaerobic metabolism resulting from decreased blood flow.

Following the induction of anesthesia but prior to aortic exposure the femoral vein of one of the extremities was exposed and control aliquots

of blood removed for study. To date these samples have been subjected to analysis for pH, Hct., $\%O_2$ sat., pO_2, pCO_2, HCO_3, CO_2, SCO_2, Lactates, Pyruvates, La/Pa ratio, and basic and acidic amino acids. In addition the refractive index of the serum was determined and from this index the total protein, total solid, pre cent total solids, water concentration and specific gravity of the serum were determined. One half hour following cross clamping of the aorta and just prior to release of the aortic clamp samples were removed and analyzed as above. Immediately following the restoration of flow, samples were again removed and sampling was continued at 5 minute intervals thereafter for periods up to one-half an hour.

Eight patients have been studied to date as outlined above. An additional patient who had a right iliac artery occlusion for 20 hours and in whom it was possible to re-establish arterial continuity was studied prior to and following the restoration of arterial flow. In one patient an aliquot of RISA was injected into the right arm immediately following aortic occlusion and 2 cc. samples of whole blood were simultaneously removed from the right arm and right femoral vein in the hope that this would furnish us with a semi quantiitative index of differential flow between the body segment above the occlusion and that below.

RESULTS

The results of the isotope experiment were as follows:

TABLE I

Time fr. injection	Arm	Leg	Ratio (L/A)
3 min.	7950 c/m	1200 c/m	15%
6 min.	5800 c/m	clotted	
10 min.	5600 c/m	3600 c/m	64
15 min.	5600 c/m	3616 c/m	64

The results of blood gas studies are outlined in figure 1. In all patients studied there was distinct evidence of anaerobic metabolism which occurred during the period of ischemia. Lactic acid and pyruvic acid levels in the venous drainage from the extremity increased significantly as did the La/Pa ratio. Per cent O_2 saturation and pO_2 declined as did pH values.

In these patients who had Dextran infusion (upper extremity) during anesthesia the degree of anaerobic change distal to the cross clamped aorta does not appear to be as great as occurs in those patients who were infused with Dextrose and Water during the operative procedure (Fig. 1).

The results of refractometric studies were outlined in the previous progress report.

Generally the specific gravity of the serum, total solids and total protein of the serum showed variable degrees of decline in all patients during aortic occlusion. Conversely, the water concentration of the serum increased during the same period of time.

The most striking change in amino acids occurred in the plasma levels of ethanolamine. In all but one instance there was an increase in the plasma level of this amino acid during the "washout" period and the rise in this ethanolamine was most pronounced in the "washout" samples of the patient who had 20 hours of occlusion. Three substances, phosphoserine, glycerophosphoethanolamine and phosphoethanolamine, all related to the metabolism of ethanolamine were observed to increase in two patients. None of these intermediates of lipid metabolism are normally present in

significant amounts in the plasma. The greatest increase in these compounds are observed in the patient who likewise had the most pronounced rise in ethanolamine. In this same patient there was a decrease in the level of glutamine and an increase in the level of glutamine acid and ammonia as well as a rise in aminobutyric acid during the "washout" period.

The changes observed in the blood from the lower extremity parallel those which are known to occur in animal shock preparations and would seem to indicate that this "preparation" is a good one for the study of metabolic changes in reversible peripheral shock in human beings.

Reversibility of "Refractory Shock"

Patients who are considered to be in a state of refractory shock are being studied by the clinical shock trauma unit. This study has been limited to those patients whose shock has not resulted from the loss of whole blood but are in a form of septic shock whether this be from gram negative or gram positive organisms. To date, all of these patients have been admitted to the unit from other areas of the hospital following a period of vasopressor therapy to which they have evidenced gradually decreasing blood pressure and urinary output in spite of increasing incriments of vasopressor drugs. All have been either oliguric or anuric for varying periods of time preceeding admission to the unit.

Each of these patients have demonstrated either acidosis, hyponatremia, hypovolemia or a combination of these derangements. Those who have been hyperthermic have had their body temperatures reduced artificially to either normothermic or hypothermic levels. Reduction to normothermic levels has been the rule.

Systematic correction of hyponatremia, hypovolemia and correction of pH combined with temperature reduction to normal has resulted in at least temporary reversal of the shock state in all instances to date, discontinuation of vasopressor therapy, re-establishment of urinary output and salvage of the patient in most instances.

[redacted]

The data on these patients is being collected and will be presented in manuscript form when a more sizable series has been accumulated.

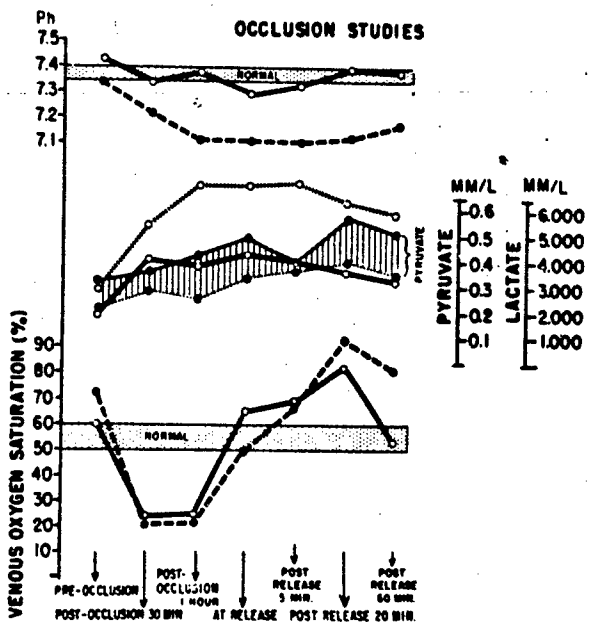

Figure 1 Ph o——o Dextran treated
●---● D/W treated

Lactate o——o Dextran treated
o---o D/W treated

Pyruvate ●——● D/W treated
●---● Dextran treated

Venous oxygen o——o Dextran treated
●---● D/W treated

Serum Refractometric Changes in Shock

The accuracy of determining total soldis and water concentration from measurement of refractive index has been well documented. The estimation of total protein concentration of the plasma by refractometry has also been advocated for years. In our laboratory we have utilized refractometric techniques to study changes in serum specific gravity, water concentration, total proteins and total solids in a variety of hypotensive patients.

One hundred and ten (110) determinations have been carried out in healthy fasting volunteers to establish control values for this laboratory. Serial determinations have been made in 40 patients in "shock" from a variety of causes. In all patients in shock there is a fall in total protein, total solids and serum specific gravity during the "shock phase" and subsequent to recovery from hypotension. Water concentration of the serum increased during the same period. As recovery occurs these changes revert to normal over a period of days. If shock becomes irreversible, there is in general a progressive fall in total proteins, total solids and serum specific gravity and a concomitant rise in serum water concentration of the serum are all significantly elevated (average 95.5 gms./100 cc.) when compared to normal (average 93.5 gms./100 cc.). Conversely specific gravity of the sera of terminal shock patients averages 1.0214 compared to the normal of 1.0248. There is no doubt that some of this change is istrogenic. The rate of Dextran, D_5W etc. in serum refractive index change is currently being evaluated.

Complete data on the above four studies are being prepared for publication.

Renal Anoxia

While awaiting completion of the renal chemistry laboratory the study of acute renal failure in dogs by means of temporary occlusion of the renal artery bilaterally over a four hour period is now in progress. In a series of 31 animals, the enzymatic alterations in ischemia of kidney tissue have been analyzed:

A. Before clamping of renal artery.

B. Immediately before clamps were released after the four hour occlusion period.

C. One week later, if animal survived (3 animals).

D. Before sacrifice, 6 to 12 weeks, when kidney function of survivors had returned to normal or had become stationary (3 animals).

In 16 dogs the enzymatic response to temporary ischemia was analyzed only after the clamping period. No follow up studies were done on these dogs. Enzymes analyzed so far are ALD, GAPDH, GDH, GOT, GPT, G-6-PDH, CDH, MDH, PGK, PGI, TIM.

The final response to temporary ischemia (4 hours) of kidney tissue in dogs showed a significant decrease in all enzyme activity except ALD. The activity of this enzyme increased in most of the experiments.

Final evaluation and statistical analysis will be done after a series of additional experiments. In this study blood content of tissue and enzyme

activity of whole blood will be determined. By this means, the total amount of enzyme activity per gram wet weight will be corrected by subtracting the amount of enzyme activity in the blood content of the tissue.

Effects of 20% Mannitol on the Kidney

Our clinical experience with mannitol in oliguria-anuria following open heart surgery and resection of aortic aneurysm demonstrated the protective effect of 20% mannitol on the kidney in the prevention of acute renal failure. This led us to the use of mannitol in patients in shock with oliguria-anuria. The charts of these patients are being reviewed to evaluate the role of mannitol in renal failure associated with shock.

B. EVALUATION OF OHP, HYPOTHERMIA, AND COMBINATION OF THE TWO AS THERAPEUTIC TOOLS

Previous work - Septic Shock: Gram negative coliform bacteremia is the most common cause of septic shock in surgical problems both elective and traumatic. The protocol was designed in an effort to study (1) the pathophysiology in this type of shock and (2) methods of management. The experimental model consisted of randomized mongrel dogs under chloralose anesthesia. Chloralose was selected since this agent does not depress reflex mechanisms, ventilation, or cardiac output. The study has been divided into three phases (1) control bacteremic shock (2) hypothermia management (3) OHP at three atmospheres.

Experimental Model: Bacteremic shock was induced by the instillation of a saline suspension of feces into the peritoneal cavity. One and a half to three grams per Kg. was found to be effective in producing the shock state within two to four hours after instillation. The shock state in many respects resembles that observed clinically. Pertinent features included the development of a hypotension accompained by a tachycardia and a hyperpnea. The hypotension occurred at the time of flooding of the blood stream by bacteria. The predominant organism isolated was E. coli and this was usually in combination with other organisms including streptococcus, pseudomonas, Aerobacter. The death rate was 88% within four hours of the sepsis and 100% within six hours. Pertinent biochemical features included a widening of the A-V oxygen difference to three times

that of normal. The oxygen consumption was relatively changed or somewhat diminished despite the extreme hyperpyrexia. A metabolic acidosis developed characterized by significant lacticidemia. The oxygen content of the arterial blood was higher than normal to the point of death. The PC_{O2} actually was lowered until just before death at which time it began to rise somewhat, but not to levels significantly above baseline. Death was characterized by a slowing of the heart rate and of the respiratory rate showing evidence of breakdown of compensatory mechanisms. At this time the blood stream was flooded with microorganisms. Hemoconcentration developed. Blood volumes were done using PAH-I^{131}. In every animal the blood volume was reduced, due to loss of plasma into the peritoneal cavity.

This preparation is similar to that observed clinically in the following respects: (1) Mixed coliform infection (2) hypotension accompained with tachycardia and hyperpnea (3) reduction in cardiac output (4) predominant E. coli organisms (5) widening of the A-V oxygen difference and little change in oxygen consumption (6) metabolic acidosis with lacticacidemia. The coincident dropping of blood pressure with flooding of the blood stream by microorganisms is a positive relationship and the shock state becomes progressively worse as the number of bacteria in the blood stream increases. The increase is due, not only to the actual entry of the organisms into the blood stream but also to the progressive failure of host defense mechanisms. Septic shock due to peritonitis is hypovolemic shock as demonstrated by

the loss of plasma into the peritoneal cavity.

Similarity from the human situation include (1) the use of homologous coliform bacteria, rather than autogenous. The former situation was attempted in animals by avulsion of the cecum. Precisely the same pathophysiologic picture developed but required 24 to 48 hours. The homologous preparation was decided upon because of the time factor (2) there has been an increasing incidence of gram positive (staphococcus aureus) invasion in peritonitis complicating the over-all picture. It was not duplicated in these experiments.

The pathophysiologic picture in these animals appears to be initiated by a reduced cardiac output likely due to decreased venous return. The decreased venous return appeared to be due in part to hypovolemia and in part to depressed peripheral vasomotor reflexes. The net result is a decreased perfusion to the tissue as demonstrated by the widening of the A-V oxygen difference. It is of interest to note that up to the point of death this widening was progressive indicating that the tissue was continuing to metabolize. Extraction of oxygen from the blood was maximal under the circumstances of these studies. The compensatory mechanisms of importance include tachycardia and hyperpnea which persists up to the point of death. Death was characterized by slowing of the heart which preceded the slowing of the respiratory rate. The measurements of the tachycardia is suggestive evidence that irreversibility in these preparations was probably not central.

Hypothermia studies: This same preparation was subjected to hypothermia to 32°C after septic shock state appeared. On the average survival rate was prolonged 2 1/2 times over that of the controls. It is noted that no other therapy was administered to the animals. The hypovolemia appeared to be arrested temporarily, but in the end was of the same magnitude as in the control animals. The striking observation of the effect of hypothermia physiologically was the inhibition of the increase of A-V oxygen difference. Soon after the low temperature level was reached, a stable period apparently developed which had been established by the shock state prior to cooling. However, within six to eight hours the progressive nature of the abnormal changes resumed and all of the animals succumbed. The net effect of hypothermia then is metabolic and one of delaying the eventual irreversibility. The conclusion with regard to hypothermia is that it serves only as a temporary stop-gap.

The effect of hypothermia in these animals basically is the same as that in humans, at least from the metabolic standpoint. However, in other respects the response is entirely different. The pressor response for example was absent in these animals as well as the urinary response.

OHP: A group of this type of preparation was subjected to OHP at three atmospheres for two hours. The mortality rate was 100% and there was no increase in the survival time. The physiologic picture was essentially similar to that of the control animals with one striking

exception. During OHP the compensatory tachycardia was inhibited and the heart rate dropped to preshock levels. The biochemical picture demonstrated some temporary arrest in its progressive pathologic nature for the first hour, but thereafter followed the pattern of the control animals. Again there was one striking feature in that the lacticidemia was much worse in the OHP dogs than in the controls. It is concluded that in the intact preparation OHP failed to alter the course of bacteremic shock.

B. Comparison of OHP in three forms of shock.

(1) Traumatic - rats were used and traumatic shock was obtained by drumming at a rate of 40-45 RPM on the Nobel-Collip drum. Approximately 800-850 turns resulted in a severe shock state but with a reasonable degree of survival. In the control animals the survival rate was less than 40%. In the OHP treated animals the survival rate was increased to 80%.

(2) Hemorrhagic Shock - The modified Fine preparation was used in which the blood pressure was kept at 30 mm Hg for 2 1/2 hours before reinfusion. In the control animals survival rate was only 13% whereas in the OHP dogs the survival rate was approximately 75%. For the effect of OHP on myocardial metabolism in hemorrhagic shock see appendix C

(3) Pulmonary Embolization - Studies in tissue hypoxia resulting from massive pulmonary embolization treated with OHP continues.

DISCUSSION

In the three forms of shock traumatic and hemorrhagic responded favorably to OHP whereas septic shock demonstrated no response whatever.

This poses some rather intriguing problems. The proposed unitarian theory of endotoxemia as being cause of irreversibility in all forms of shock regardless of primary ideology may be subject to question as suggested by these experiments. If endotoxemia had been responsible for irreversibility in all three forms of shock then there should have been no response to OHP in the traumatic and hemorrhagic shock dogs. The fact that they did, does not negate the role of endotoxins in shock state at some point. However, the matter of endotoxemia being primarily responsible for irreversibility will require more extensive investigation.

Another conclusion of these studies is that merely increasing the availability of oxygen to the tissue is not the answer to the management of shock problems, particularly septic shock. More definitive therapy is necessary as is generally appreciated. The role of OHP may well be that similar to hypothermia; that is, adjunctive.

FUTURE PLANS

(A) Septic Shock: We propose to repeat the above studies this time applying the established regimen in the management of septic shock, specifically appropriate antibiosis. Also, in this regard it has been demonstrated that OHP inhibits the growth of gram negative organisms. Since the septic shock animals under OHP failed to demonstrate any significant degree of survival other factors with regard to OHP in this situation must be considered. One of the mechanical factors of import-

ance is the transmission of pressure from the skin surface into the peritoneal cavity. The body resists very strongly any pressure changes and it is quite likely that while the concentration of oxygen in the peritoneal cavity is elevated the accompanying pressure elevation may be smaller. Preliminary studies to date have indicated that while the chamber pressure is at three atmospheres, the pressure in the peritoneal cavity is actually only one atmosphere. In effect then, what happened in the previous studies is simply that the high oxygen concentration was provided to the organisms resulting in a more luxuriant growth and likely an increased elaboration of endotoxins. This appears to be borne out by virtue of the fact that the biochemical picture in the OHP septic shock dogs was much worse than that of the septic shock control dogs. In this line, future studies are planned in which the peritoneal cavity will be widely opened after septic shock has been induced and before the animal is placed in the chamber. This will provide for equilibration of the chamber pressure in the peritoneal cavity. The clinical overtones of this activity might be as follows: The treatment of septic shock in the intact individual by OHP may be to no avail. However, emergency surgery in acute peritonitis might be safer in an OHP chamber.

Hemorrhagic shock and extracorporeal perfusion: It has been previously demonstrated under our previous contract that the standard Fine preparation improved temporarily after one hour of total bypass. However,

after bypass was discontinued, all of the animals died. It is proposed to use the modified preparation developed at the University of Maryland and repeat these studies with a view toward obtaining long term survivals.

Additional information of both published and unpublished material Section IV, Part B can be found in appendix D.

C. THE ROLE OF IMMUNO-BACTERIOLOGICAL PROBLEMS IN SHOCK

(1) Following up studies on immuno-bacterial defense mechanisms: A comprehensive survey of immuno-bacterial defense mechanisms in the human during shock has been made in the past y[illegible]. This has been reported. The conclusions gained from this study were that the human being is very susceptible to bacterial infection as a result of shock and the susceptibility lasts for a period of 12 to 18 hours. After successful resuscitation 24 - 48 hours later the patients will show a rebound reaction with a presumed high resistance against infection, although this has not yet been clinically proven. The pattern of depression followed by rebound is similar to the pattern which occurs when a marginally immunized individual is given a booster shot. Initially after the booster shot there is a depression in the amount of the circulating antibodies and then this is followed by the amnéstic response with a rebound. The similarity of this pattern and to those noted in animals when they were exposed to endotoxic shock has prompted us to feel that the patterns observed in humans may be due to the absorption of some noxious antigen. Surveying the literature indicates that not only endotoxin can produce a depression in natural antibody, complement, and phagocytosis, etc. with a rebound reaction, but also such things as symozan can produce this. The next logical step is to investigate the possibility of absorption of antigen and this has been undertaken

in two ways. The first method involves the obtaining of plasma from patients in profound shock and the immunization of rabbits with this plasma, paired with immunization of rabbits with normal human plasma. After a period of time, the rabbits are investigated for their bactericidal titer against E. coli and if this should be stimulated by the shock plasma but not stimulated by the normal plasma, it would indicate that there is an antigen in the shock plasma which has been causing the patterns that we observed in the humans. Preliminary results on a series of rabbits seem to indicate that this is so, although this work is in progress and cannot yet be considered valid. A second method will be to test the plasma directly by in vitro methods in the test tube for the presence of an antigen similar to endotoxin. We are in the process of doing this by preparing a sensitive inhibition of hemagglutination test with endotoxin that we have prepared from our own strain of E. coli. The test is just being developed and when it is perfected we will begin to use it on the patients.

(2) The studies that were mentioned previously involved phagocytosis by the peripheral polymorphonuclear leucocytes. An even more important group of phagocytes in the body involved in bacterial clearance and in endotoxin detoxification are those of the reticuloendothelial system, i.e., the fixed phagocytes. Fine has shownthat the phagocytes in the spleen will ingest endotoxin and will digest, and metabolize it liberating free radioactive phosphorus if the endotoxin has been tagged prior to its administration. This means that the reticuloendothelial system not only removes the endotoxin from the circulation but also metabolizes and

neutralizes it. Plasma which is obtained after passage through the spleen no longer has endotoxin properties. To follow this up, we have begun to investigate the reticuloendothelial system during shock using radioiodinated serum albumin which has been alkalinized and heat-treated so that it is aggregated in molecular groups of 11. This preparation will then be phagocytized by the reticuloendothelial system as shown by Bennaceraf and his group, but if it does not cross the species barriers it will not be antigenic, i.e., if human serum albumin is used, humans will not show an allergic response to this as shown by Taplin and Wagner and others who have used this material. This is a safe preparation for use in humans and we are presently investigating it in the dogs during shock because it has never been studied during shock and there may be some untoward reactions. In the dogs we have accumulated data which we are about to publish which indicates that there are two phases of reticuloendothelial system function alteration occuring with hemorrhage and shock. The first phase occurred with hemorrhage alone when the dog is not in profound shock. In this phase there is an inhibition of uptake of the albumin by the reticuloendothelial system, i.e., the phagocytosis is reduced. However, the metabolism as measured by the release of free radioiodine or the reaccumulation of radioiodine in the circulation, is not effective until shock actually occurs. Once shock occurs, phagocytosis is decreased even more but the metabolism is now decreased and this is fairly specific for the shock syndrome. This would fit in with the findings that we found with complement wherein hemorrhage alone in the human will result in the decrease of

complement, but an immediate restoration after the patient is given blood. If there is shock, the restoration of blood volume does not result in a restoration of complement until 12-24 hours later. The same seems to be true of the reticuloendothelial system regarding metabolism, when shock is added to hemorrhage. We plan to get this data together within the next month or so and submit it for publication and also to use it so that we may begin to use the heat-treated serum albumin to study shock patients. It will be impractical as we have found with the dog to try to follow the patient during the resuscitation phase because the administration of blood and fluids which are necessary to save the patient will interfere with the radioactive iodine study by dilution factors, etc.. What we plan to do is to study the patients immediately after they have been resuscitated and see if they have a lasting effect on the reticuloendothelial system and how long this lasts. In addition we hope to study volunteers with hemorrhage without shock. This work on the RES system will complement the work we have done on complement natural antibody and phagocytosis by the peripheral phagocytes.

(3) The Shock Trauma Unit will be getting both an experimental and a clinical hyperbaric oxygen chamber. We are, therefore, interested in the bacteriological aspects of hyperbaric therapy. Members of this group have shown the beneficial effects of hyperbaric oxygen on hemorrhagic shock. In lieu of the presumptive evidence that we may be accumulating for the role of endogenous endotoxin in hemorrhagic shock, we wondered if the effects noted by Attar and his group could be due not only to beneficial effects on the host but also possibly to inhibition of the sources of endo-

toxin, i.e., the fecal flora. As a result, we performed in vitro studies using purified E. coli cultures as well as mixed fecal cultures and comparing the results in an incubator at atmospheric oxygen with a result at various time intervals under hyperbaric oxygen as far as growth of bacteria is concerned. We found that hyperbaric oxygen completely inhibits the growth of E. coli in a certain preparation wherein we poured 100 colonies into pour plates and compared them after eight hours of hyperbaric oxygen. This is strictly a bacteristatic effect for the colonies will appear once taken out of hyperbaric oxygen. We wondered if this would be of benefit in fecal peritonitis as we found a similar result with mixed cultures. We, therefore, noted the results by Blair as well as some of our own results on hyperbaric oxygen effects on fecal peritonitis with septic shock and noted that there was little or no beneficial effect, i.e., the animals died just as rapidly as did controls. We ran cultures on these animals and found that they had a steadily increasing septicemia beginning one-half hour to one hour after installation of feces. We, therefore, wondered if the reason why the results we noted in vitro with inhibition were not of any value was because the oxygen tension in the peritoneal cavity where the bacteria were contained was not sufficient to produce inhibition. We then studied the oxygen tension in the colon and the oxygen tension in the closed peritoneal cavity and found that the oxygen tension in the colon was in equilibrium with the tank, i.e., any bacteria in the colon lumen would be inhibited. The oxygen tension in the peritoneal cavity, however, was in equilibrium with the tissue never getting above 1 atm of oxygen, even under 3 atm external environment for 4 - 5 hours. We thus

concluded that intercolonic bacteria would be inhibited, but interperitoneal bacteria or bacteria that had made their way into the tissues would not be. Inhibition of intercolonic bacteria may play some role in the beneficial effect on hemorrhagic shock. We then left the peritoneal cavity open on the animals after installation of feces and got a completely different picture indicating the beneficial effect of hyperbaric oxygen by its inhibition of bacteria. Instead of a steadily increasing septicemia, there was a septicemia which decreased and produced almost total clearance, or at least partial clearance in a period of 3 - 5 hours after hyperbaric oxygen treatment. The animals then went on with this very severe preparation to an increasing septicemia and died in eight hours as compared to five hours for controls not treated with hyperbaric oxygen or were left at room temperature. This occurred in a significant group of dogs and is statistically valid. We compared this with studies in fluid media in the rate of growth of bacteria under hyperbaric oxygen conditions, and found that there was a definite decrease in E. coli growth in broth between 2 and 5 hours which correlated with the dog's clearance in the blood stream. We hope to apply this clinically when we get a tank by venting the peritoneal cavity or venting the abscess cavities in case of fecal peritonitis and using the hyperbaric oxygen not only to help the host withstand the effects of shock more, but also to inhibit the bacteria directly. We are devising a method of venting the peritoneal cavity with a vent under local anesthesia which will allow relatively sterile technic and drainage at the same time. We then wondered about the application of hyperbaric oxygen under conditions

such as perhaps burns where the bacteria are presumably on the surface of the skin and would be reached by the oxygen. We then surveyed a large number of bacteria including staphylacoccus, pneumococci Klebsiella, pneumonia, and pseudomonas pyocyaneus. In general, we found that the gram positive organisms were less susceptible to hyperbaric oxygen especially those which actually resided in the upper respiratory tract. The effects of Klebsiella and the effects of pneumococci were minimal compared to the effects on E. coli. The same was true for staph, although there was inhibition here. When it came to pseudomonas, however, we got a completely different picture, i.e., for the first 3 - 4 hours after exposure, hyperbaric oxygen and fluid cultures pseudonomas grew better under three atm. of oxygen than it did under room tension as opposed to all the other organisms. This means that pseudomonas is resistant to hyperbaric oxygen and we believe that this has been found by others. Therefore, its use in burns if pseudomonas is involved would be of no benefit as far as inhibition of the bacteria are concerned. Further studies to clarify this before it is used clinically are indicated. We also intend to see if there are alterations in bacterial metabolism which result in alteration of sensitivities to antibiotics as a result of hyperbaric oxygen.

This encompasses our immediate plans with regard to the shock program as far as research protocol are concerned in the bacteriological section. (See Appendix E).

D. EVALUATION OF AESTHETIC PROBLEMS AND AGENTS IN OHP

Study in this area is awaiting the installation of the OHP Chamber.

E. ANIMAL RESEARCH TO FURTHER AUGMENT THE STUDY AND TREATMENT OF SHOCK

Enzyme Studies

With the possibility in mind to expedite routine examination of specific activities of enzymes in tissues, tests were done to compare protein determinations by the colorimetri nethod (Folin-Ciocalteu) by a spectrophotometric method (Warburg and Christian) and by spectrophotofluorometry. It was found that brain extracts prepared with distilled water would give good results both with spectrophotometry and colorimetry. Spectrophotofluorometric measurements deviated from those obtained with the other two methods. With .1 molar phosphate buffer as extracting medium good agreement was observed between spectrophotometry, colorimetry and fluorometry. However, since the calibration curves for spe trophotometry and fluorometry differed from preparation to preparation we could use the colorimetric method for protein determinations in organ extracts.

Tests on survivorship of rats in drum shock were continued. The tolerance to shock increases when OHP is breathed. Since exposure to oxygen above certain limits is lethal, these tests were continued and it was found that a narrow margin exists here. A significant therapeutic effect was found with three atmospheres OHP. The effect of two atmospheres OHP and of oxygen of one atmospheres was small and was stat-

istically not significant. The experiments for tolerance to OHP in shock animals are being continued.

Changes of enzyme activity in brain of rats in air and in OHP control vs. shock. Aldolase is statistically significantly increased in air. This increase is wiped out when OHP at three atmospheres is breathed but no change occurs at lower pressures. Glucose 6-phosphate dehydrogenase appears to be unaffected in shock with or without subsequent application of OHP. Diaphorase was tested without application of OHP and it was found that shock causes no significant difference in this enzyme. A few experiments were also done with lactic dehydrogenase and malic dehydrogenase with DPNH as donor and they indicate that shock preparations show a decrease of these enzymes confirming earlier results obtained in this laboratory with dichlorophenolindophenol as acceptor. Because in these experiments diaphorase is supposed to act as hydrogen transporting enzyme and since diaphorase is not affected in shock these few experiments on lactic and malic dehydrogenase with DPNH as donor support our view that the activities of dehydrogenase are decreased and that the decrease cannot be ascribed to changes in diaphorase.

The decrease of specific activity of enzymes could be caused by increase in nonenzymatic protein in shock brain preparations

or by structural alterations in the dehydrogenase. Brain protein electrophorograms were made from control and shock rats and dog indicate possible qualitative difference between patterns with control and shock preparations whose differing amounts of drumming were compared. The shock preparations appeared to be optically more dense than those of controls. There was a poorly defined, more rapidly migrating peak on the controls. This became progressively less discernible with increasing amount of tumbling. These experiments are being continued with a new electrophoretic technique.

Chemotherapy in Hemorrhagic Shock.

Studies in hemorrhagic shock using trial chemotherapy are continuring. To date, using our standard modified Fine technique, a significant increase in survival rate has been noted in animals pretreated with (a) quanidine sulfate, a potent anti-hypertensive drug and (b) neomycin sulfate, an antibiotic to sterilize intestinal flora is restudied as a control for the coronary occlusion study of cardiogenic shock (See "3" below).

Preliminary studies of effects of d-aldosterone on hemorrhagic shock has been reported in the Final Technical Report on the Origin and Utilization of Ammonia in Shock (DA-49-007-674). A more intensive study was made to determine the major effects of this drug on electrolyte balance, metabolism and blood gases in the normotensive dogs and dogs subjected to hemorrhagic shock. (See Appendix F)

Cardiogenic Shock.

With the possibility in mind that sterilization of the gut would increase the survival rate of dogs subjected to massive coronary occlusion and resulting infarction, pretreatment with neomycin sulfate was administered. On a percentage basis, twice the number of pretreated dogs survived as compared to the control dogs.

Utilizing the same technique of coronary occlusion aldosterone was studied as a means of increasing the survival rate. Dogs pretreated with aldosterone 20 minutes pre-occlusion resulted in a 100% mortality. Those infused with aldosterone immediately post-ligation all survived. This study looks most promising and is being pursued with interest. (See Appendix G)

F. SHOCK PATIENTS STUDIED TO DATE

Breakdown of Patients Studied Under Grant Prior to Unit Opening
(Prior to 3-14-63)

Shock Classification	Patients	Source of Admission				Days		Improved	Expired
		Wd	AR	RR	Other	Total # Days	# Days Studied		
Septic	4	2	2			5	1.2	2	2
Hemorrhagic	8	4	4			33	4.2	6	2
Traumatic	16	2	14			48	3.0	15	1
Potential (Impending)	6	6				6	1.0	6	
Miscellaneous a. Mesenteric thrombus or infarcted bowel	3	2	1			10	3.3	2	1
Total	37	16	21			102	2.7	31	6

(64)

BREAKDOWN OF PATIENT ADMISSIONS TO SHOCK TRAUMA UNIT
3-14-63 to 12-1-63

Classification	Patients	Source of Admission				Days		Outcome	
		Wd	RR	AR	Other	Total # Days	Average # Days	Improved Transferred	Expired
Septic	19	13	1	5		74	3.9	12	7
Hemorrhagic									
G.I.	12	7	1	4		93	8.1	6	6
Other	6	4		2		12	2.0	3	3
Cardiac									
Cardiac Arrest	2	1			1	4	2.0		2
Other	2		2			6	3.0		1
Traumatic	3			2	1	24	8.0	3	
Potential Shock (Critically Ill)									
Mult. Frac.	6	3	1	1	1	60	10.0	6	
Post Op.	3	3				21	7.0	3	
Mult. Fistulae	1	1				18	18		1
Blunt Trauma	1	1				14	14	1	
Bleeding	1	1				15	15	1	
Occlusion Studies (not adm. to unit)	8								
Total	64	36	5	14	3	341	8.18	35	20

PATIENTS UNDER CONTROL STUDIES

		Source of Admission Ward	Days Total # Days	Days Average # Days
Admitted to Unit For complete study	10	10	3.5	3.5
Not Admitted to Unit				
a. Amino acid analysis	8			
b. Refractometry	106			
c. Bacterial Defense Mechanisms	10			
d. Lipid analysis	5			
Total	139	10	3.5	3.5

SUMMARY

PATIENTS STUDIED - SHOCK TRAUMA UNIT

Patients admitted to study prior to 3-14-63	37
Patients admitted to study subsequent to 3-14-63	64
Patients admitted to unit for control studies	10
Patients studied as controls - Not admitted to unit	129
Total	240

V. CRITIQUE

The University of Maryland School of Medicine has been awarded by United States Army Contract No. DA-49-193-MD-2229, dated January 1, 1962, funds for initiation of a study of shock in man. This was the first such award made by an Army contract.

Almost all of the experiential problems relevant to this program seem to have evolved from two major factors. These factors are (1) original nature of the study (a scientific study of shock in man), and (2) unprecedented design of the study.

The above factors which have allowed freedom for creative research have also imposed problems in organization and programming. Guidelines appropriate for management or resolution of these problems were unavailable since none had been established previously.

Architectural Renovations:

Architectural renovations were necessary in order to provide space and facilities for conducting the study.

Because clinical patient areas had to be converted into essential shock laboratories, an existing hospital bed shortage was further compounded. An ideal area for the study could not be established until additional space was provided. This space was subsequently designated in the Third Floor Bressler Building for future occupancy by February 1963. However, the aforementioned space did not actually become available for occupancy until November 1963.

The reestablishment of some laboratories from the hospital to the Bressler area has eventuated in a general overall improvement. Completion of the moving phase, in transition at present, is expected to result in further improvement.

The clinical shock laboratories located in the hospital were not completed and made active until March 1963. During the above mentioned interval, makeshift studies were carried out in the wards of the surgical floors. These studies were pilot in nature and in many instances were hampered by immobility of either the shock patients or the necessary equipment to conduct studies at the patients' bedside.

Inadequate Numbers of Personnel

In the beginning, there were inadequate numbers of personnel available for the scope of the study project. This shortage was demonstrable in all categories: scientists, technicians and nurses. The training of unqualified technicians and time spent searching for qualified personnel partially diverted the research effort.

Improving the caliber of the supervisory cadre has now rewarded the Unit with a competent staff of technicians supporting a complex shock laboratory on a 24 hour around-the-clock basis.

Since September 1963, the nursing staff has finally stabilized. Previous to that time there had been little stabilization since many nurses interviewed for the program felt that their background did not equip them to handle such critically ill patients. This problem has been solved by presenting a series of lectures on trauma and shock, as well as entering these nurses in our

cardiac surgery nursing program wherein they are taught the various techniques of acute patient care, resuscitation procedures and monitoring techniques. Interest has further been stimulated by allowing these nurses in their free time to enter an electronics program so that they will be better equipped to handle and control the monitoring devices.

Professional personnel in the quality and type required has posed great difficulty. Qualified scientists interested in clinical shock research are scarce. Most scientists of the Ph. D. level are already oriented along certain lines and it is difficult to request their assistance in an alien field. Competition with lucrative industrial positions further hampers recruitment of desirable people. We are pleased to report that Dr. Sadayoshi Hashimoto, a surgeon and biochemist, will again rejoin our staff before the year is out. The loss of Dr. Gerhard Laudahn, who returned to Germany, was an unexpected setback. Unforeseen events such as this have hampered the biochemical protocol. Active proselytizing in the Ph.D. biochemical field has made available two qualified men interested in shock, providing resources are available for their employment. Employment of a physiologist is essential.

Inadequate Funds

Renovation of old laboratory areas and the establishment of new laboratory areas has necessarily diverted funds channeled for other uses. It has been necessary to further acquire funds for additional renovations through private resources. These deficits, to some extent, futther compromised the Unit in that established laboratory areas could not be fully

utilized until delays in funding and contracting were overcome in order to establish the new areas previously described.

One important phase of the shock preogram will soon be initiated since funds have been made available and contracting bids let for installation of the research hyperbaric experimental chamber this coming year (January 1964).

To summarize, the deficiencies in space, personnel and funding have been outlined. Many of these problems have been solved and much progress has been made in attaining a successful operating clinical shock unit. We now have a substantial flow of shock patients. Some monitoring equipment has been installed and nurses and technicians have been trained in its operation. Shock information is now accumulating and we hope that valuable data will ensue. We are adding more complex studies as the unit progresses.

The described deficiencies and problems already encountered seem inevitable in this new type of hospital operation. We feel justified in looking forward to the year ahead when the progress made so far can be expanded successfully to carry out the functions of the protocol called for by the contract.

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APPENDIX A

THE PATIENT MONITORING SYSTEM FOR THE CLINICAL SHOCK UNIT

One of the major demands of any research protocol is the collection and storage of data in a format that will permit rapid recall and display as well as easy reduction and calculation. In the clinical research unit of the Shock study, the patient monitor system used to collect research data must be capable of providing an indication of the patient's condition at all times.

The monitor system to be used in the Clinical Shock Unit will provide a continuous indication of the condition of the patient and accumulate data on phenomena occurring in conjunction with shock. An alarm system capable of alerting hospital personnel, automatically by radio communication when any one, or combination, of eight patient events deviates from present limits, will be attached to the unit. The entire patient monitor system will be coordinated with additional equipment used for computer handling of the data. This additional equipment will include a tape recorder and an analog to digital converter and will be incorporated at a future time.

The patient monitor will provide four output displays. An eight channel oscilloscope and an eight channel pen recorder will display and record such events as ECG, EEG, pressure wave patterns, heart sounds and similar AC events. A twelve channel, multipoint null balance recorder and eight panel mounted meters (alarm meters) will record and display data such as pulse rate, body temperatures, respiratory rate, and blood, respiratory gases and urine chemistries.

Inputs to the patient monitor will be located at these major parts of the system. Junction boxes will be located onthe patient's bed for connecting the required patient transducers to the system. A cable carrying all of the patient signals will connect the mounted junction boxes on the bed to an appropriate input connector located on the wall near the patient's bed. This will permit the patient's bed to be moved to any point in the hospital where an input connector is located without removing any of the transducers, such as ECG leads or pressure gauges, from the patient. Signals from these transducers will enter the monitor at either the multipoint recorder or at the input couplers of an eight channel pre-amplifier, depending upon their component frequencies. A second data entry point will be located on the rear of the twelve channel multipoint recorder and alarm meter panel. This entry point will receive signals from pH meters, CO_2, O_2 and similar gas or chemical analyzers. Patient signals such as temperatures will also enter at this point via the bed mounted junction boxes. Tachometers and integrator circuits will be attached to the pre-amplifier outputs to provide heart rate, mean pressure, etc., to be entered at the meter-multipoint recorder input . The third location of information entry will be on the front of the multipoint recorder and will receive coded information concerning the treatment given to the patient and other pertinent comments entered by the personnel in the Trauma Unit andwill print directly on the record containing the patient's condition, as indicated by the multipoint recorder. This part of the system requires the use of the IBM electric typewriter discussed below.

The Honeywell Electronik 15 is a null balance, twelve channel recorder. Its purpose in the patient monitor will be to record slow changing events such as heart rate, respiratory rate, body temperature and body chemistries. This unit will print one channel every 30 seconds or every 5 seconds, (switch selectable), thus cycling all twelve channels every 6 minutes or every minute. Normally, the paper speed will be either 5, 10, 15 and 20 inches per hour, or 30, 60 90 and 120 inches per hour depending on the print cycle speed listed above. In order to correlate the information on the Honeywell chart with the data recorded on the eight channel pen recorder with regard to time of event occurrence, a printer which automatically stamps the date and time on the recording paper, will be attached to both recorders. The Honeywell Electronik 15 recorder was selected for this system because of its accuracy, input characteristics, paper calibration, size and competitive cost.

The IBM " Selectric" typewriter was incorporated in the system because it has no moving carriage. This is essential since the paper coming from the multi-channel print wheel on the Honeywell recorder will be fed into the typewriter and from the typewriter back to the take-up reel of the Honeywell recorder. Used in this capacity, the IBM typewriter becomes an electronic printer capable of printing a code concerning patient treatment directly on the record paper, which contains the information on the patient's response to that treatment. The IBM will alter the accuracy of the Honeywell paper speed. Since the time and date will be printed directly on the record paper, this will not be detrimental to the system. Some minor modifications will be necessary to the face plate of the Honeywell recorder and the IBM typewriter

will have to be adjusted so as not to clamp the paper. These modifications can be done here at the hospital and will not render either the recorder or typewriter useless in their normal capacities.

A Sanborn Monitor Scope, Model 769, was selected for use as the eight channel oscilloscope required by the patient monitor because it provided the greatest display area at the lowest cost, and could be located at a remote part of the Trauma Unit, where it would not interfere with the normal activity surrounding the patient. The viewing area measures 13 inches high and 10 inches wide. A laminated polaroid filter in front of the CRT face prevents reflection and serves as a safety glass. The simplicity of the circuitry employed will enable the scope to provide trouble-free displays.

Most of the electronic equipment will be housed in the console containing the eight channel pen recorder. The equipment manufactured by Offner was chosen for several reasons. The pen writers provide multi-color ink and rectilinear writing in a relatively small space. This will allow the outputs to be color coded, immediately displayed in a linear form and recorded on a medium which is less expensive than any other available. The Offner preamplifier system was selected, independent of the pen recorder, because it provides the greatest flexibility consistent with a high degree of accuracy and small size. The function of any preamplifier can be changed by changing its input coupler. The input couplers cost an average of sixty dollars. Other types of systems would require the changing of complete channels costing several hundred dollars.

The alarm meters are manufactured by International Instruments.

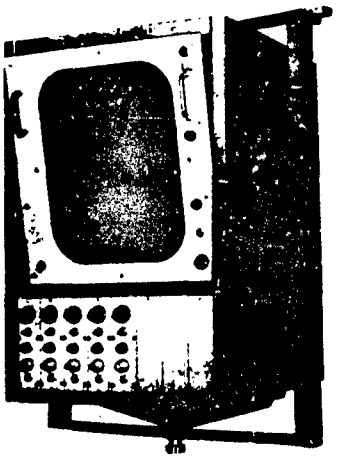
They were selected because their geometry provides the greatest amount of indication in the smallest amount of panel space. Two levers on the meter are used to set the limits for the event being monitored. If the meter pointer goes outside of these limits, relays are activated which trigger an alarm system.

The alarm system consists of audio-visual alarm devices on the patient monitor panel and a radio transmitter which transmits the alarm to six pocket sized radio receivers carried by hospital personnel. The alarm system can be wired to fire when any one event or any logical series of events deviates from the set limits. The alarm transmitter can also transmit a 500 cycle tone needed to trigger the alarm receivers when any pulse shaped event, such as a pressure pulse or ECG, occurs.

The alarm transmitter which provides an amplitude modulated carrier of 27.255 megacycles was designed and built in the hospital's electronics laboratory and is capable of delivering 30 watts of power.

All of the major equipment necessary for the patient monitor system has been purchased with the exception of the Honeywell multipoint recorder and the Offner pen recorder. Purchase of these items has not yet received approval of the granting agency for the Shock Research project. The patient monitor system will be operational within thirty days after the delivery of these two components.

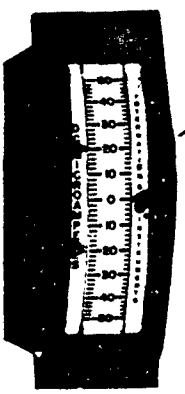
CEILING MOUNT

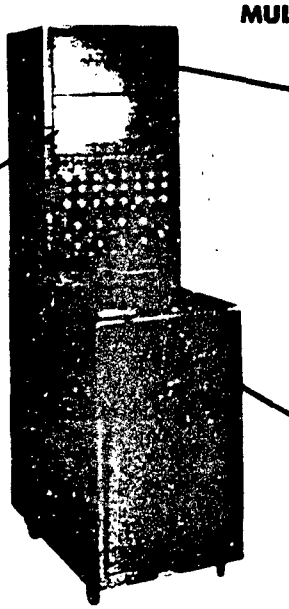


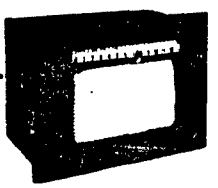
Eight channel scope

EKG, EEG, pressures,

MULTIPOINT RECORDER



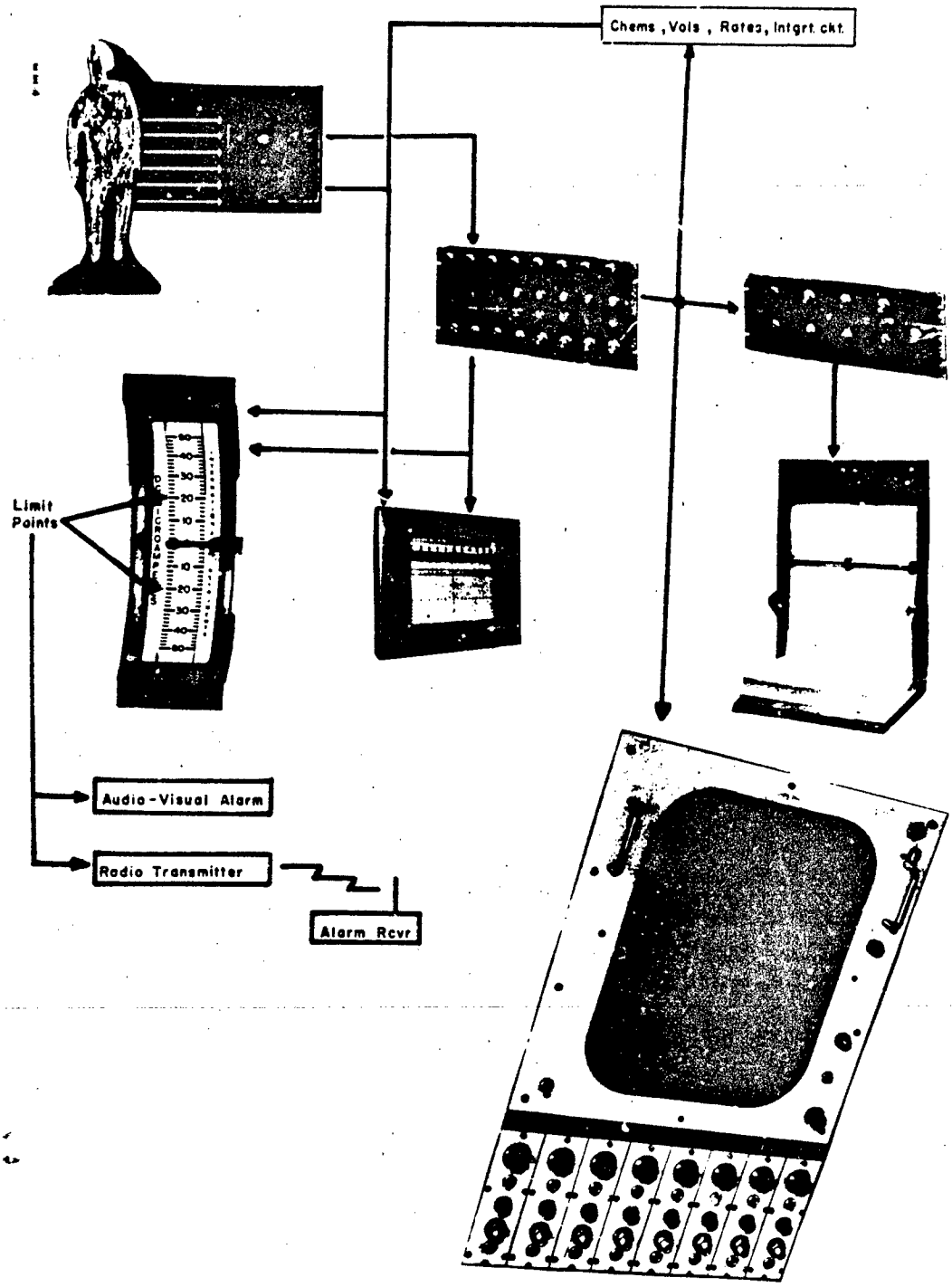


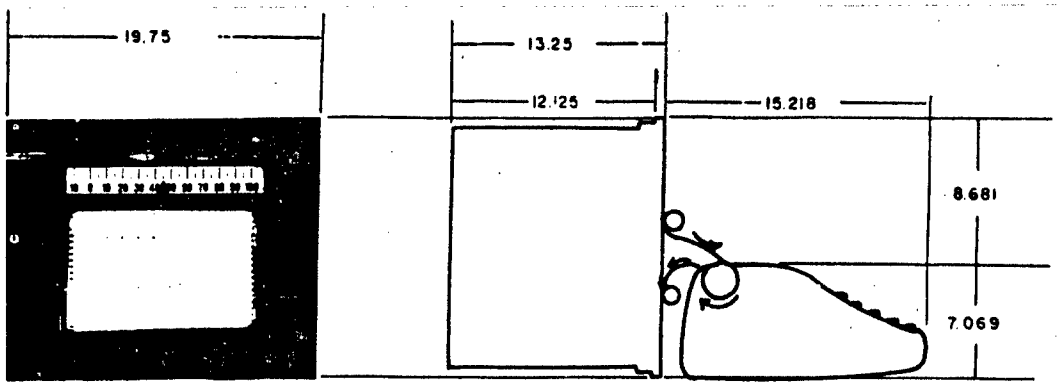


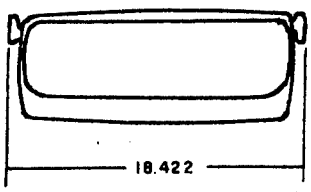
12 points
temp.
pulse rate
resp. rate
fluid vols.
chem. values

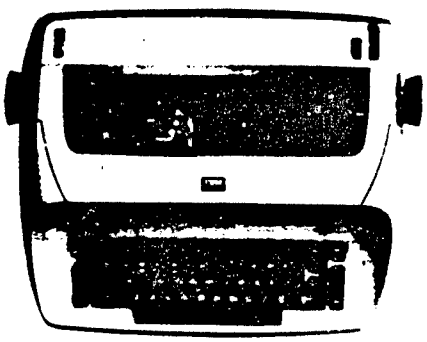
Eight channel, rectilinear, ink recorder for recording events being monitored on the eight channel scope.

Eight alarm meters for phenomena displayed on multipoint recorder.


Chems , Vols , Rates, Intgrt. ckt.
Limit Points
Audio-Visual Alarm
Radio Transmitter
Alarm Rcvr







IBM-Honeywell Patient Treatment Recorder

APPENDIX B

STUDIES OF AMINO ACID METABOLISM DURING SUBLIMINAL PERFUSION

Amino acid analyses carried out in this laboratory have been directed toward a quantitative description of clinical shock in terms of impaired cellular metabolism. Our initial efforts have been the designing of an *in vivo* model for the study of cellular metabolism during subliminal perfusion.

During surgical occlusion of the abdominal aorta, immediately below the renal artery, the impaired perfusion of the lower extremities distal to the occlusion site provides an ideal preparation for the study of anoxic muscle metabolism relatively independent of systemic physiological response. Accordingly, we have undertaken the amino acid analysis of plasma obtained from the femoral veins of patients undergoing abdominal aortic occlusion in the course of their surgery[1]. Eight such patients have been studied in this manner.

The amino acid analyses were carried out on a Beckman Model 120-B Amino Acid Analyzer. The amino acids which were determined are listed in Table I. Also listed in Table I are several other metabolites which were determined along with the amino acids due to their ninhydrin color reaction. Plasma tryptophan was not assayed because it is unstable under the conditions employed in the preparation of plasma for amino acid analysis. Asparagine and glutamine are eluted as a single component in the analyzer. Therefore, the values obtained for glutamine are to be taken as the sum of asparagine and glutamine concentrations. This does not lead to a serious error, in that asparagine has been reported to be present in human serum at a level of only 0.04 um (1) whereas glutamine is present at a level approximately 14 times

as great, 0.57 um. The technique of sampling consists of exposing the femoral vein at the time of laparotomy for patients undergoing abdominal aortic surgery. During the surgical procedure, 30 cc aliquots of blood are taken from the femoral vein in the following sequence: I. During anesthesia, after femoral vein cut down, II. One-half hour after aortic occlusion, III. Immediately before aortic clamp is released, IV. Immediately after clamp is released, V. Five minutes after release, VI. Twenty minutes after release, VII. Sixty minutes after release, and Control - brachial artery. The plasma levels of most of the amino acids determined by us are in close agreement with the values reported by Stein and Moore[1]. Our values for methionine, isoleucine, and phenylalanine are somewhat higher than those reported by these investigators. Several of the metabolites listed in Table I were not found at a significant level in the pre-occlusion plasma samples. In most cases their presence is indicated by only a slight deviation in the otherwise linear base-line. This may be secondary to anesthesia. Normal plasma studies are being determined to track down this discrepancy.

Amino acid analyses were performed on eight pre-occlusion plasma samples. The values obtained are shown in Table II. The quantities are tabulated as micromoles of amino acid/3.3 ml of plasma since the preparation of plasma for amino acid analysis is such that the amount of the final preparation analyzed represents a 3.3 ml aliquot of the original plasma. This volume, therefore, was used throughout these studies as a matter of convenience.

The results of these occlusion analyses are tabulated in Tables III to VIII inclusive. An increase in the plasma level of ethanolamine during washout was observed in five of the six cases studied and was most pronounced in the first case (Bush), which was a 20 hour occlusion study. Three metabolites metabolically related to ethanolamine were also determined by the amino acid analyzer. These are phosphoserin glycerophosphoethanolamine, and phosphoethanolamine. None of these intermediates of lipid metabolism are normally present in plasma at levels exceeding 0.01 micromole per 3.3 ml. It is interesting to note that the levels of these metabolites increased significantly during washout in two of the cases studied (see Tables III and IV) and that the most significant increase was found in the case with the most pronounced rise in plasma ethanolamine. This case also revealed a decrease in the level of glutamine and an increase in the levels of glutamic acid and ammonia. The level of aminobutyric acid was also elevated during the washout.

Some patients in clinical shock have been studied with regard to plasma amino acid levels in an attempt to relate these findings to metabolic changes which occur as a result of subliminal perfusion. The most apparent change in the plasma amino acid levels of each of the patients studied was a decrease in the level of total plasma amino acids. Increases in total plasma amino acids have been reported in the literature, presumably as a result of extravasation and the accompanying hemoconcentration. It is not clear how such a mechanism might increase the levels of circulating

amino acids. On the contrary, the elevated levels of catecholamines which have been reported in clinical shock should bring about, via adrenocortical hormone release, an increase in gluconeogenesis, thus lowering the level of circulating amino acids. The normal plasma level of total amino acids in micromoles per 3.3 ml, was found to be 10.8 ± 1.6. This value represents the sum of the concentrations of all of the metabolites listed in Table I with the exception of urea and ammonia, the concentrations of which were found to be so variable in the patients studied that they interferred with the interpretation of the more subtle changes observed in the concentrations of the amino acids. The range of total plasma amino acids in the patients studied was 3.8 to 8.4, all significantly below the normal range.

Another change which was observed in each of the patients studied was a decrease, relative to the total amino acid concentration, in the level of glutamine and an increase in the relative level of ammonia. Since anaerobic metabolism is associated with a rise in the plasma levels of organic acids, principally lactic and pyruvic, the resulting acidosis would be common to all of the clinical shock patients, regardless of etiology.

The primary function of renal glutaminase is conservation of the alkali reserve. (glutamine- - - - - → glutamate + NH_4^+). The regeneration of glutamine is endergonic, requiring ATP as the energy source. During anoxia the limited supply of ATP probably prevents significant regeneration. For this reason the ratio of plasma ammonia to plasma glutamine concentrations serves as a measure of the physiologic response to the acidosis of anoxia.

The value of this ratio in the normal plasma samples was found to be 0.1[illegible] ± 0.02.

In the three patients studied the ratios were 0.53 (Wilson), 0.36 (Williams), and 0.57 (Whitford). The significance of this ratio is more apparent when one considers that the highest ratio (0.57) was obtained from a patient whose plasma ammonia level was normal (Whitford) (0.40 um). Thus, this method of measuring the compensatory mechanism for conservation of the alkali reserve seems to be relatively independent of the activity of the Krebs-Henseleit cycle for detoxification of ammonia and the efficiency of renal function in the clearance of urea. The levels of plasma urea in these three patients ranged from somewhat below normal (Williams = 10.8 um) to uremic (Wilson = 158.4 um), with an elevated urea level in the case of Whitford, 32.0 um. The normal urea level was found to be 13.6± 0.4 um/3.3 ml plasma.

A number of changes in plasma amino acid levels were observed in these patients which reflect the individual etiology and response to shock. For example, in the case of Wilson the most notable changes besides uremia and azotemia (reflecting renal failure) were a significant relative decrease in the level of glucogenic amino acids, particularly alanine and glycine. This can be ascribed to an accelerated gluconeogensis in response to ACH elaboration. Also noted in this case was a relative increase in the plasma levels of taurine, valine, and phenylalanine. Relative increases in plasma taurine and phenylalanine was also noted in the case of Whitford. Also

noted in the Whitford plasma was a significant relative decrease in the level of proline. These changes are not clearly understood and require further study.

The case of Williams is particularly interesting, in that it represents a relatively uncomplicated case of hypovolemic shock resulting from acute extravasation. The most pronounced change in this patient's plasma was a significant relative decrease in the level of alanine, probably reflecting a gluconeogenetic response to ACH, accompanied by accelerated transamination to produce pyruvic acid in compensation for tissue anoxia. (alanine + alpha-ketoglutarate - - - → pyruvate + glutamate). This mechanism is further supported by the observation of a relative increase in the plasma level of glutamate. The relative decrease in plasma serine and increase in plasma glycine suggest an acceleration of the hepatic serine hydroxy-methylase reaction: (serine + tetrahydrofolate - - - - → glycine + hydroxymethyltetrahydrofolate.) Such a mechanism could increase the pool of one-carbon units and bring about the slight relative increase noted in the plasma level of methionine. The plasma level of ethanolamine was significantly increased in this patient, constituting 2% of the total plasma amino acids on a molar basis. This metabolite is present in normal plasma only at very low levels and possibly represents the hepatic catabolism of serine: (serine - - - → 2 aminoethanol.) The decreased relative levels of tyrosine and phenylalanine possibly reflects the renal mobilization of these amino acids for catecholamine synthesis.

The results of the plasma amino acid analyses on clinical shock patients is presented in Table IX.

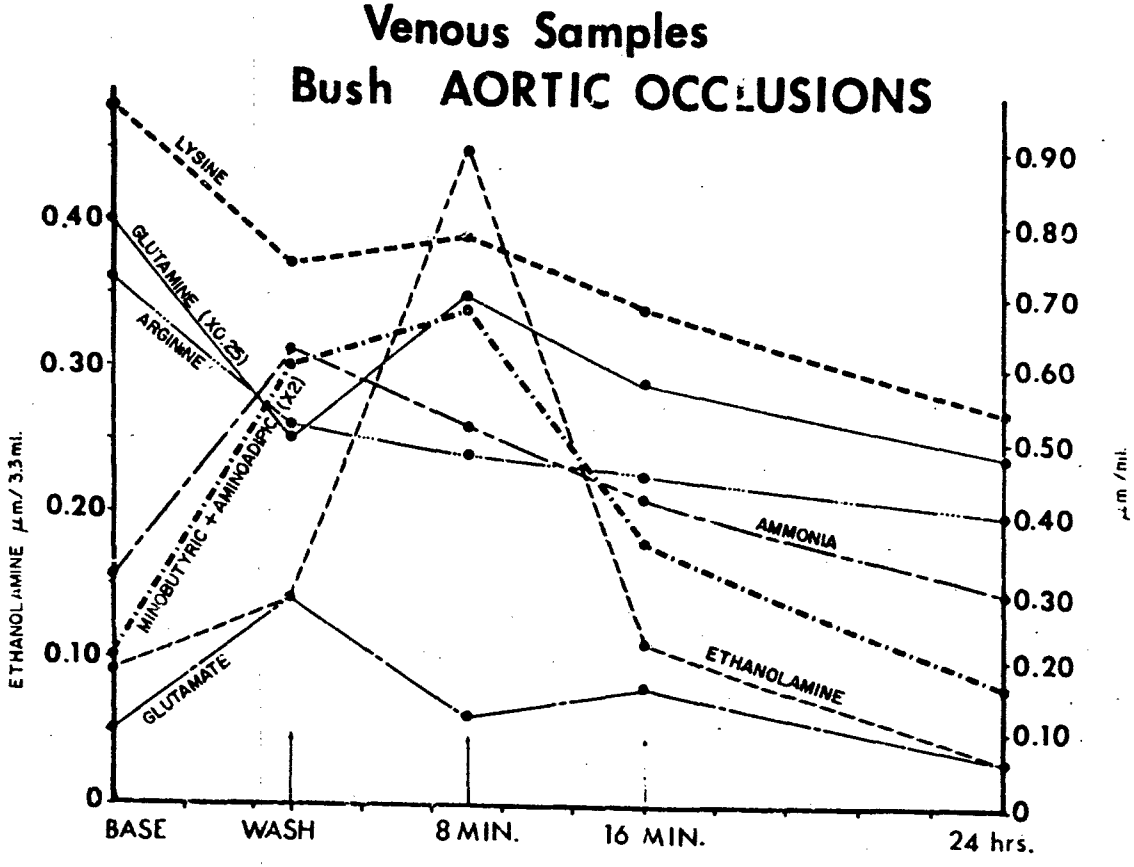

Venous Samples
Bush AORTIC OCCLUSIONS
LYSINE
GLUTAMINE (X0.25)
ARGININE
MINOBUTYRIC + AMINOADIPIC (X2)
GLUTAMATE
AMMONIA
ETHANOLAMINE
ETHANOLAMINE µm/ 3.3ml.
µm/ml.
0.40
0.30
0.20
0.10
0
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
BASE
WASH
8 MIN.
16 MIN.
24 hrs.

Fig. 1.

TABLE I

NINHYDRIN-POSITIVE METABOLITES IN HUMAN SERUM

Alanine	Leucine
Alpha-Amino-n-butyric Acid	Lysine
Ammonia	Methionine
Arginine	Ornithine
Citrulline	Phenylalanine
1/2 Cystine	Proline
Ethanolamine	Serine
Glutamic Acid	Taurine
Glutamine	Threonine
Glycine	Tyrosine
Histidine	Urea
Isoleucine	Valine

TABLE II

PLASMA AMINO ACIDS*

u moles 3/3 ml plasma

SUBJECT	#1	#2	#3	#4	#5	#6	#7	#8	Ave.	Range
Taurine	0.13	0.13	0.19	0.24	0.17	0.24	0.18	0.17	0.18	± 0.06
Urea	14.00	15.85	14.80	(25.02)	18.65	16.36	10.91	13.93	16.19	± 6.83
Threonine	0.41	0.42	0.37	0.46	0.32	0.33	0.27	0.33	0.36	± 0.10
Serine	0.32	0.41	0.33	0.22	----	0.28	0.26	0.32	0.31	± 0.10
Glutamine	2.31	2.22	1.61	(1.04)	2.08	2.21	1.61	2.30	1.92	± 0.39
Proline	0.47	0.40	0.41	----	----	0.52	0.51	0.48	0.46	± 0.06
Glutamic Acid	0.16	0.11	(0.05)	0.20	----	0.13	(0.06)	0.08	0.11	± 0.09
Citrulline	0.14	0.10	(0.05)	0.14	0.10	0.07	0.08	0.08	0.09	± 0.05
Glycine	0.64	0.84	0.72	0.90	0.76	0.63	0.58	0.57	0.70	± 0.20
Alanine	1.09	1.28	1.17	1.30	1.22	1.61	0.83	1.07	1.20	± 0.41
Amino butyric acid	0.05	0.06	0.05	0.10	(1.24)	0.05	0.04	0.03	0.20	± 1.04
Valine	0.85	0.84	0.75	0.82	0.78	0.86	0.49	0.69	0.76	± 0.10
1/2 Cystine	0.07	0.07	0.13	0.08	(0.20)	0.11	0.08	0.10	0.10	± 0.10
Methionine	0.21	0.23	0.28	0.26	0.32	0.17	(0.10)	0.17	0.22	± 0.10
Isoleucine	0.39	0.42	0.47	0.46	0.54	0.35	0.18	0.34	0.39	± 0.15
Leucine	0.02	0.01	0.01	0.04	0.02	0.01	0.03	0.02	0.02	± 0.02
Tyrosine	0.24	0.16	0.22	0.24	0.26	0.14	0.11	0.15	0.19	± 0.07
Phenylalanine	0.21	0.17	0.26	0.20	0.27	0.14	0.10	0.17	0.18	± 0.09
Ornithine	0.16	0.13	0.09	0.32	0.09	0.12	0.13	0.18	0.15	± 0.17
Ammonia	0.40	0.44	0.36	0.62	0.24	0.65	0.30	0.48	0.44	± 0.21
Lysine	0.59	0.54	0.48	0.64	0.34	0.46	0.34	0.48	0.48	± 0.16
Histidine	0.24	0.24	0.16	0.20	0.12	0.18	0.14	0.15	0.18	± 0.06
Arginine	0.33	0.24	0.18	0.16	----	0.23	0.17	0.24	0.22	± 0.11

* Samples drawn pre-Abdominal Aortic Occlusion

TAB: . III
AMINO ACID STUDIES

ABDOMINAL AORTIC OCCLUSION

Date: February 5, 1963

Patient: Bush

	#I	#II	#III	#V	#VI	#VII
Phosphoserine	- - -	0.03	0.11	0.04	0.04	- - -
Glycerophosphoethanolamine	- - -	- - -	- - -	0.02	- - -	- - -
Phosphoethanolamine	- - -	- - -	0.02	0.02	- - -	- - -
Taurine	0.19	0.08	0.17	0.18	0.21	0.03
Urea	14.80	15.47	14.67	15.47	12.93	25.87
Methionine Sulfoxides	- - -	0.02	0.01	0.01	0.02	0.01
Aspartic Acid	- - -	- - -	- - -	0.02	0.01	0.01
Threonine	0.37	0.11	0.26	0.29	0.12	0.20
Serine	0.33	0.17		0.26	0.21	0.19
Asparagine	- - -	- - -	1.29	- - -	- - -	- - -
Glutamine	1.61	0.76		1.40	1.17	0.96
Sarcosine	- - -	- - -	- - -	- - -	- - -	0.06
Proline	0.41	0.43	0.56	0.54	0.37	0.30
Glutamic Acid	0.05	0.12	0.14	0.11	0.08	0.03
Citrulline	0.05	0.03	0.04	0.06	0.05	0.04
Glycine	0.72	0.28	0.55	0.60	0.53	0.32
Alanine	1.17	0.39	1.10	1.14	0.93	0.77
a-Aminoadipic Acid	- - -	0.04	0.04	- - -	0.03	- - -
a-Amino-n-butyric Acid	0.05	0.14	0.11	0.17	0.06	0.04
Valine	0.75	0.35	0.53	0.65	0.53	0.81
Half Cystine	0.13	0.02	0.05	0.09	0.05	0.05
Methionine	0.28	0.12	0.17	0.17	0.17	0.17
Isoleucine	0.47	0.17	0.35	0.40	0.34	0.40
Leucine	0.01	0.004	0.01	- - -	0.01	0.01
Tyrosine	0.22	0.09	0.15	0.17	0.14	0.17
Phenylalanine	0.26	0.14	0.23	0.23	0.21	0.22
B-Aminoisobutyric Acid	- - -	- - -	- - -	- - -	0.04	- - -
y-Aminobutyric Acid	- - -	0.05	- - -	- - -	- - -	- - -
Ornithine	0.09	0.05	0.08	0.06	0.06	0.11
Ethanolamine	0.09	0.27	0.14	0.45	0.11	0.03
Ammonia	0.36	0.41	0.62	0.52	0.42	0.29
Lysine	0.48	0.17	0.37	0.39	0.34	0.27
Histidine	0.18	0.08	0.14	0.14	0.14	0.11
Arginine	0.18	0.04	0.13	0.12	- - -	0.10

TABLE IV
AMINO ACID STUDIES

ABDOMINAL AORTIC OCCLUSION

Date: February 2, 1963

Patient: Newcomer

u moles

	#I	#II	#III	#IV	#V	#VI
Phosphoserine	0.01	- - -	0.04	0.005	0.01	0.01
Glycerophosphoethanolamine	- - -	- - -	0.06	- - -	- - -	0.01
Phosphoethanolamine	- - -	- - -	- - -	- - -	- - -	0.02
Taurine	0.12	0.30	0.37	0.23	0.37	0.48
Urea	12.51	21.2	19.57	12.52	21.11	20.48
Methionine Sulfoxides	0.02	0.05	0.01	0.03	0.03	0.03
Aspartic Acid	0.01	0.007	0.01	0.01	0.01	0.01
Threonine	0.23	0.45	0.42	0.22	0.34	0.38
Serine	0.11	0.54	0.58	0.24	0.36	0.52
Glutamine	0.52	1.37	1.95	1.35	1.97	1.91
Proline	0.49	0.80	0.77	0.40	0.60	0.75
Glutamic Acid	0.10	0.11	0.21	0.07	0.14	0.14
Citrulline	0.07	0.09	0.09	0.05	0.09	0.11
Glycine	0.45	0.87	0.83	0.46	0.78	0.77
Alanine	0.65	1.86	2.42	1.57	2.14	2.41
a-Aminoadipic Acid	0.17	0.24	0.27	0.07	0.16	0.16
a-Amino-n-butyric Acid	0.05	0.07	0.07	0.03	0.06	0.06
Valine	0.41	0.58	0.70	0.44	0.72	0.82
Half Cystine	0.04	0.001	0.16	0.40	0.06	0.17
Methionine	0.13	0.18	0.17	0.10	0.17	0.18
Isoleucine	0.23	0.33	0.39	0.22	0.40	0.46
Leucine	0.02	0.047	0.04	0.02	0.03	0.03
Tyrosine	0.12	0.20	0.20	0.11	0.19	0.21
Phenylalanine	0.10	0.19	0.22	0.19	0.21	0.27
B-Aminoisobutyric Acid	- - -	0.019	0.02	- - -	- - -	- - -
Ornithine	0.16	0.28	0.26	0.14	0.21	0.21
Ethanolamine	0.01	0.01	0.05	0.02	0.04	0.03
Ammonia	0.31	0.34	0.59	0.30	0.60	0.38
Lysine	0.32	0.56	0.58	0.34	0.54	0.52
Histidine	0.10	0.22	0.24	0.14	0.21	0.20
Arginine	0.08	0.18	0.18	0.09	0.15	- - -

TABLE V
AMINO ACID STUDIES

ABDOMINAL AORTIC OCCLUSION

Date: February 2, 1963

Patient: Peylon

	#I	#II	#III	#IV	#V
Taurine	0.17	0.19	0.19	0.17	0.18
Urea	18.65	20.67	18.72	18.29	21.05
Methionine Sulfoxides	0.01	0.01	0.01	- - -	- - -
Aspartic Acid	0.01	- - -	0.02	- - -	- - -
Threonine	0.32	0.52	0.50	0.48	0.45
Serine	- - -	- - -	- - -	0.51	0.43
Glutamine	2.08	2.64	2.41	2.44	2.41
Proline	- - -	0.49	- - -	0.44	0.44
Glutamic Acid	- - -	0.06	- - -	0.05	0.13
Citrulline	0.10	0.09	0.10	0.08	0.09
Glycine	0.76	0.84	0.84	0.77	0.78
Alanine	1.22	1.57	1.47	1.38	1.39
a-Amino-n-butyric Acid	1.24	0.10	0.25	0.08	0.08
Valine	0.78	1.13	0.80	1.08	1.20
Half Cystine	0.20	0.27	0.34	0.25	0.11
Methionine	0.32	0.41	0.38	0.37	0.21
Isoleucine	0.54	0.73	0.72	0.74	0.69
Leucine	0.02	0.03	0.03	0.03	0.02
Tyrosine	0.26	0.29	0.27	0.27	0.28
Phenylalanine	0.27	0.34	0.32	0.33	0.32
Ornithine	0.09	0.18	0.19	0.16	0.20
Ethanolamine	0.01	0.03	0.03	0.005	0.02
Ammonia	0.24	0.50	0.58	0.41	0.44
Lysine	0.34	0.97	0.72	0.72	0.73
Histidine	0.12	0.22	0.31	0.22	0.22
Arginine	- - -	0.31	0.46	0.30	0.27

TABLE VI
AMINO ACID STUDIES

ABDOMINAL AORTIC OCCLUSION

Date: March 18, 1963

Patient: Kovach

	#I	#II	#III	#IV	#V	#VI	#VII	Control
Glycerophosphoethanolamine	- - -	- - -	- - -	0.01	0.01	0.01	0.008	- - -
Taurine	0.24	0.28	0.31	0.30	0.34	0.37	0.33	0.24
Urea	16.33	17.04	18.35	16.80	18.27	15.71	14.92	12.51
Methionine Sulfoxides	- - -	- - -	- - -	0.02	- - -	- - -	- - -	- - -
Aspartic Acid	- - -	- - -	- - -	0.01	- - -	- - -	- - -	- - -
Threonine	0.33	0.30	0.32	0.28	0.31	0.27	0.25	0.18
Serine	0.28	0.26	0.26	0.25	0.28	0.25	0.22	0.16
Glutamine	2.21	2.33	2.47	2.02	2.15	0.92	1.53	1.02
Proline	0.52	0.47	0.70	0.48	0.52	0.50	0.46	0.35
Glutamic Acid	0.13	0.06	0.08	0.12	0.17	0.17	0.11	0.19
Citrulline	0.07	0.06	0.06	0.06	0.05	0.06	0.05	0.05
Glycine	0.63	0.64	0.67	0.59	0.56	0.55	0.48	0.34
Alinine	1.61	1.99	2.53	2.17	2.36	2.10	1.94	1.15
a-Amino-n-butyric Acid	0.05	0.05	0.05	0.05	0.04	0.04	0.03	0.02
Valine	0.86	0.77	0.87	0.80	0.86	0.85	0.79	0.57
Half Cystine	0.11	0.10	0.10	0.09	0.10	0.09	0.08	0.06
Methionine	0.17	0.12	0.11	0.10	0.12	0.09	0.06	0.06
Isoleucine	0.35	0.31	0.33	0.30	0.35	0.32	0.27	0.19
Leucine	0.01	0.02	0.03	0.02	0.02	0.01	0.01	0.007
Tyrosine	0.14	0.11	0.13	0.12	0.13	0.12	0.10	0.07
Phenylalanine	0.14	0.13	- - -	0.12	0.16	0.14	0.12	0.08
Ornithine	0.12	0.12	0.13	0.13	0.13	0.12	0.10	0.06
Ethanolamine	0.03	0.02	0.03	0.03	0.04	0.03	0.03	0.02
Ammonia	0.65	0.43	0.49	0.55	0.57	1.29	0.54	0.45
Lysine	0.46	0.44	0.50	0.48	0.51	0.43	0.39	0.20
Histidine	0.18	0.22	0.13	0.22	0.22	0.22	0.20	0.08
Arginine	0.23	- - -	0.23	0.20	0.25	0.19	0.16	0.10

TABLE VII
AMINO ACID STUDIES

ABDOMINAL AORTIC OCCLUSION

Date: March 28, 1963

Patient: Quail

	#I	#II	#III	#IV	#V	#VI	#VII
Taurine	0.18	0.18	0.17	0.12	0.18	0.15	0.21
Urea	10.91	11.27	11.84	8.21	12.71	10.00	13.31
Methionine Sulfoxides	0.02	0.02	0.02	0.01	0.02	0.01	0.02
Threonine	0.27	0.26	0.26	0.18	0.26	0.21	0.25
Serine	0.26	0.23	0.41	0.19	0.24	0.21	0.23
Glutamine	1.61	1.58	1.48	1.14	1.55	1.29	1.48
Proline	0.51	0.48	0.52	0.36	0.51	0.40	0.49
Glutamic Acid	0.06	0.05	0.05	0.06	0.09	0.07	0.10
Citrulline	0.08	0.07	0.07	0.06	0.07	0.06	0.08
Glycine	0.58	0.54	0.51	0.39	0.53	0.42	0.49
Alanine	0.83	0.90	1.00	0.73	1.02	0.79	0.95
Aminobutyric	0.04	0.03	0.04	0.02	0.03	0.02	0.03
Valine	0.49	0.49	0.49	0.36	0.54	0.46	0.63
Half Cystine	0.08	0.07	0.08	0.06	0.08	0.06	0.07
Methionine	0.10	0.09	0.09	0.06	0.08	0.06	0.05
Isoleucine	0.18	0.17	0.19	0.15	0.21	0.17	0.25
Leucine	0.03	0.02	0.02	0.02	0.02	0.02	0.02
Tyrosine	0.11	0.10	0.11	0.08	0.11	0.09	0.11
Phenylalanine	0.10	0.09	0.11	0.08	0.12	0.10	0.12
Ornithine	0.13	0.12	0.12	0.08	0.13	0.10	0.10
Ethanolamine	0.01	0.01	0.02	0.02	- - -	0.01	0.01
Ammonia	0.30	0.31	0.29	0.23	0.35	0.25	0.28
Lysine	0.34	0.31	0.33	0.24	0.33	0.29	0.31
Histidine	0.14	0.13	0.15	0.10	0.15	0.13	0.15
Arginine	0.17	0.18	0.15	0.12	0.16	0.13	0.13

TABLE VIII
AMINO ACID STUDIES

ABDOMINAL AORTIC OCCLUSION

Date: April 2, 1963

Patient: Hoffman

	#I	#II	#III	#IV	#V	#VI	#VII
Phosphoserine	- - -	- - -	0.005	0.007	0.005	0.003	- - -
Glycerophosphoethanolamine	- - -	0.01	0.010	0.011	0.011	0.013	0.014
Phosphoethanolamine	- - -	- - -	0.008	- - -	- - -	- - -	- - -
Taurine	0.17	0.25	0.33	0.03	0.27	0.31	0.33
Urea	13.93	14.93	17.03	15.09	13.09	13.91	12.79
Methionine Sulfoxides	0.03	0.03	0.03	0.03	0.02	0.03	0.03
Aspartic Acid	- - -	0.01	0.007	0.007	- - -	- - -	- - -
Threonine	0.33	0.34	0.44	0.36	0.30	0.33	0.34
Serine	0.32	0.30	0.38	- - -	0.26	0.27	0.30
Glutamine	2.30	2.50	3.59	- - -	2.26	2.54	2.59
Proline	0.48	0.55	0.72	0.64	0.47	0.53	0.55
Glutamic Acid	0.08	0.10	0.09	0.11	0.07	0.07	0.07
Citrulline	0.08	0.07	0.09	0.08	0.07	0.07	0.06
Glycine	0.57	0.61	0.79	0.68	0.55	0.62	0.62
Alanine	1.07	1.24	1.80	1.46	1.21	1.51	1.53
Aminobutyric	0.03	0.03	0.04	0.02	0.02	0.02	0.02
Valine	0.69	0.76	0.99	0.86	0.75	0.79	0.80
Half Cystine	0.10	0.11	0.16	0.13	0.11	0.09	0.12
Methionine	0.17	0.18	0.24	0.21	0.18	0.19	0.17
Isoleucine	0.34	0.37	0.54	0.47	0.41	0.43	0.44
Leucine	0.02	0.02	0.02	0.02	0.01	0.02	0.02
Tyrosine	0.15	0.16	0.21	0.18	0.15	0.17	0.16
Phenylalanine	0.17	0.18	0.25	0.20	0.19	0.21	0.21
Ornithine	0.18	0.22	0.23	0.24	0.19	0.22	0.20
Ethanolamine	0.02	0.04	0.05	0.06	0.04	0.04	0.05
Ammonia	0.48	0.50	0.47	0.57	0.38	0.40	0.34
Lysine	0.48	0.56	0.62	0.65	0.55	0.62	0.60
Histidine	0.15	0.18	0.21	0.23	0.20	0.23	0.22
Arginine	0.24	0.27	0.29	0.31	0.27	- - -	0.25

TABLE IX

AMINO ACID STUDIES

PLASMA LEVELS DURING CLINICAL SHOCK

MILLIMOLAR CONCENTRATION

Subject	Normal Average	#1	#2	#3
Taurine	0.04	0.06	0.03	0.12
Urea	4.52	48.00	3.27	9.70
Threonine	0.13	0.06	0.06	0.03
Serine	0.11	0.06	0.03	0.03
Glutamine	0.69	0.39	0.48	0.21
Proline	0.13	0.09	0.12	0.06
Glutamic Acid	0.04	0.03	0.06	0.01
Citrulline	0.04	0.02	0.06	0.01
Glycine	0.22	0.09	0.24	0.06
Valine	0.26	0.18	0.18	0.12
Half Cystine	0.02	0.02	0.01	0.06
Methionine	0.07	0.03	0.06	0.02
Isoleucine	0.12	0.06	0.09	0.06
Tyrosine	0.06	0.03	0.03	0.03
Phenylalanine	0.06	0.06	0.03	0.09
Ornithine	0.04	0.03	0.05	0.02
Ethanolamine	0.00	0.00	0.04	0.00
Ammonia	0.13	0.21	0.18	0.12
Lysine	0.17	0.10	0.11	0.07
Histidine	0.07	0.04	0.06	0.03
Arginine	0.09	----	0.06	----

Subject #1 - Renal Failure
#2 - Hemorrhagic Shock
#3 - Septic Shock

APPENDIX C

THE EFFECT OF HYPERBARIC OXYGENATION ON THE MYOCARDIAL METABOLISM IN EXPERIMENTAL HEMORRHAGIC SHOCK - UNPUBLISHED DATA

(I) Myocardial Phosphorylase Activity and its Relationship to Catecholamine in the Adrenals

Sadayoshi Hashimoto, and R Adams Cowley

Our previous studies in experimental hemorrhagic shock induced in mongrel dogs by the standard shock technique have demonstrated that oxygen breathing at a pressure of three atmospheres absolute showed a significant benefit in markedly reducing mortality in the treatment of dogs in hemorrhagic shock[1]. Simultaneously after pressurization an increased partial pressure of oxygen in the muscle and the liver in shock has been demonstrated.

Oxygen at high partial pressures has been shown to have a harmful effect on the metabolism of mammalian tissue in vitro or on individual enzyme systems[2-7]. From the systematical studies on the effect of oxygen at elevated tension on tissue respiration and on the activity on the various enzymes, Dickens[8] and Stadie et al[9,10] have concluded that many enzymes, particularly those dependent on sulfhydryl groups for activity, are more or less easily inactivated by oxygen pressures of one atmosphere or more; on the other hand, many other enzymes are completely resistant to the toxic action of oxygen.

Rall and Sutherland first demonstrated the presence of phosphorylase in the myocardium[11]. This enzyme, phosphorylase, catalyzes the reaction: glycogen + inorganic phosphate = glucose-1-phosphate. Dog heart muscle phosphorylase provides an examples of a phosphorylase

which exists in both the a (active in the absence of adenosine-5-phosphate) and b (active only in the presence of adenosine-5-phosphate) form. Recently it was reported that epinephrine and other sympathomimetic amines caused an increase in the activity of phosphorylase a of the rat myocardium [12] and these findings have focused on the relationship between integrated enzyme activity and mechanical action of the heart.

It is generally accepted that the adrenal medullary hormone of a normal animal does not play a role in the maintenance of blood pressure [13, 14]. On the other hand, the response of the adrenal medulla to abnormally low level of blood pressure has been extensively studied and they revealed that the induction of hypotension was accompained by an increase in the adrenal secretion of epinephrine [15-17]. Glaviano et al reported that the adrenals consistently demonstrated an amazing capacity to secrete continuously pressore hormones under the severe stress of progressing circulatory failure [18].

These results have led us to consider the possibility that phosphorylase in the myocardium and catecholamine in the adrenals might be interacting in experimental hemorrhagic shock.

The intermediary metabolism of the myocardium in shock has to the best of our knowledge found little attention. Mostly information was gained by the application of anoxia to the myocardium in vitro and the conclusions were that what had been seen in anoxia ought to have occurred in shock.

We considered the prevalence of anaerobic glycolysis in shock and assumed that OHP increases the oxygen concentration in the cells. These experiments deal with the effect of hyperbaric oxygenation (OHP) on the changes in phosphorylase activity of the myocardium and catecholamine content in the adrenals in experimental hemorrhagic shock.

Methods

Randomly selected mongrel dogs were employed for this study. The animals were separated into three groups : 1) control, 2) hemorrhagic shock and 3) pressure group. The procedures in the hemorrhagic shock and OHP groups were identical to those previously reported. The samples were taken at the following stages: after 2 1/2 hours shock in the second group and immediately after the depressurization of the third group.

The animals were anesthetized with intravenous administration of 1 ml/Kg. of pentobarbital. Immediately after the chest was opened, approximately 500 mg. of the apex part of the beating left ventricular wall was quickly excised and placed in an alcohol dry ice slurry previously prepared and the frozen tissue was weighed immediately. Simultaneously the animals were laparotomized and both sides of adrenals were excised. The neighboring fat tissues were removed. No frozen technique for this sample was performed.

Phosphorylase determinations were made according to method of Cori and Illingworth[20] as follows. It has been shown that the force of contraction of the heart can be depressed by pentobarbital without significant lowering of phosphorylase activity[19].

Therefore an influence of the anesthetic agent on the enzymatic activity could be disregarded. The frozen tissue was homogenized for one minute in an Omni-mixer containing 30x v/w of 0.01 M NaF-0.002 M ethylene-diamine tetracetic acid (EDTA), disodium salt After this procedure, according to the modified method of Haugaard et al[21], the 1:30 extract was diluted 5 times with NaF-EDTA edium. A 0.5 ml. sample of this diluted extract (1:150) was incubated with 0.5 ml. of 0.033 M glucose-1-phosphate, 2 per cent glycogen (with and without 0.002 M AMP) for five minutes at 30C.

The inorganic phosphate formed during the incubation was measured by the modified isobutanol method of Takahashi's[22]. The specific activity was expressed as phosphorylase units per mg. protein according to the equation of Cori et al[23]. Protein was determined by the method of Folin ciocalteau.

The catecholamine determinations were made according to the modified method of Euler[24]. The tissue was weighed and homogenized with an aliquot of 0.025 N H Cl under cooling. Then five per cent trichloroacetic acid was added and well stirred. After centrifuged under 3500 r.p.m., the supernate was filtered off. Lipids, if present, and the trichloroacetic acid were removed by subsequent repeated extraction with ether. To the clear filtrate 10-50 mg. of ascrobic acid per liter and aluminum sulfate to 0.1 per cent was added. The pH was adjusted to slightly over seven with 1N NaOH. The precipitate after centrifuging was washed with water and dissolved in 2 N H_2SO_4. The solution was diluted so that it corresponded to 20 ml. per gram and pH adjusted to 3.5 with 1N NaOH. To this solution

four volumes of 95 per cent ethanol was added, and the precipitate allowed to settle down over night in the refrigerator. After filtering, the clear filtrate was concentrated in vacuo to 5-15 ml. The colorimetric determinations were done using a Beckman spectrophotometer according to the method of von Euler and Hamberg[25]. The content of catecholamine in adrenal glands was expressed by ug. per gram of moist tissue.

Results and Discussions

The experiments reported in Table 1 demonstrate clearly that the actively contracting dog heart muscle has a high content of phosphorylase. The values for the phosphorylase ratio of 40.8 per cent indicates that phosphorylase is present predominantly in the a form. If one assumes that phosphorylase a is 65 per cent active in the absence of adrenosine-5-phosphate, the value of 40.8 per cent for the phosphorylase ratio corresponds to a content of 63 per cent of the total phosphorylase. This is close to the figure of 72 per cent reported by Cori (1956) for rabbit heart.

Table 1 illustrates that the cardiac phosphorylase a activity (percentage of total) in normal open chest dogs is 40.8± 2.9 P.E.M. (N=10). In the shock group its activity was 27.1± 2.0 P.E.M. (N=10) and therefore decreased to a greater extent if compared to control group. In the shock group treated with OHP, the activity is 34.4± 4.0 (N=6), and increased significantly when compared to shock group and almost returned to the normal range.

However, the corresponding phosphorylase units with and without adenylic acid in each group did not show any noticeable changes.

Apparently, at the terminal stage after two and one-half hours of shock, accumulation of lactic acid lowers the pH, depressing phosphorylase a content. If one assumes that OHP provides enough oxygen to show Pasteur Effect working, which would result in a lowered content of lactic acid in the OHP group compared to shock, then the phosphorylase activity seems to be increased. These findings support the conclusion that considering the effect of OHP, the decrease of phosphorylase activity induced by hemorrhagic shock is not irreversible and the integrity of enzyme system which activate cardiac phosphorylase is not impaired by hemorrhagic shock.

Hess et al concluded that hypotension per se is not responsible for the decrease in the activity of phosphorylase a seen after the vagal stimulation[21]. However, this does not apply here because the method by which hypotension was induced was different from hemorrhagic shock. Klarwein et al[26] reported that when coronary circulation was not maintained. active phosphorylase increased, then diminished in heart muscle during ventricular and atrial fibrillation and during ventricular tachycardia. This could be explained on the basis of activation of the two enzymes which catalyze the conversion of the phosphorylases[27].

Table 2 represents that the content of adrenaline, noradrenaline and total catecholamine in the shock group significantly decreased compared with controls and the values obtained in shock with subsequent OHP group have returned close to the normal range. Expressed in terms, of adrenal medullar secretion the decreased content of catecholamine in the adrenals has result in the increased release into the peripheral circulation.

These results agree with the reports showing that the adrenal medulla of dogs in hemorrhagic shock was observed to continue to secrete epinephrine at very low levels of mean blood pressure, characteristic of terminal shock[18].

Lund reported that an increase in both epinephrine and norepinephrine levels occurred in the adrenal venous blood of dogs subjected to short periods of hemorrhagic shock[26]. Manger et al[27] and Watts and Bragg[28] reported significant increases in levels of epinephrine in peripheral blood of dogs in hemorrhagic shock.

The changes in the catecholamine content in the adrenals are in agreement with these adrenal and peripheral studies.

Levenson et al[29] stated that the glycogen deposition occurs in the face of a presumably elevated level of epinephrine and led to the suggestion that in shock there is a defect of epinephrine activation of phosphorylase as a consequence of the ischemia.

However, in connection with them, concerning the decreased activity of phosphorylase in hemorrhagic shock in spite of the increased secretion of catecholamine and the decreased content in the adrenals, it appears that the action of catecholamine on the irreversible hemorrhagic shock is negligible as far as phosphorylases are concerned. On the other hand, since the administration of OHP in hemorrhagic shock increase the catecholamine content of the adrenals it has been suggested that in OHP treated shocked animals the secretion of catecholamines was decreased. It is not clear whether a cause and effect relationship exists between the changes in enzyme activity and the increase in muscle contractions. It follows from our results that no noticeably augmented

action of catecholamines on the phosphorylase activity in hemorrhagic shock has been observed. This is compatible with the fact that normal dog heart has a high concentration of phosphorylase and epinephrine causes an increase in the activity of phosphorylase a of the myocardium.

As is shown in Table 3 and Figure 1, so correlation between the content of catecholamine and phosphorylase activity are demonstrated. This was most interesting findings.

In some instances of shock group the phosphorylase values of 37.4 per cent and 26.9 per cent have been seen, and these values were accompained by relatively high catecholamine content. These values well explained the findings compared to the catecholamine content in adrenals as shown in Table 3. This might explain that such a high value appears in the shock group but not in the irreversible state. Also 17 per cent survival rate in our control experiment supports this suggestion[1].

Summary

The effect of hemorrhagic shock with and without OHP on the phosphorylase activity of dog cardiac muscle has been studied in connection with investigations into the role played by catecholamine in adrenals, a hormone figuring in the activation of cardiac phosphorylase.

1) Phosphorylase activity decreases in experimental hemorrhagic shock compared to controls and the OHP treatment restores the activity close to normal. This indicates that the hypoxic-anoxic decrease of cardiac phosphorylase activity is not irreversible and the administration

of OHP in hemorrhagic shock does not show adverse effect on this enzyme.

2) The content of adrenaline and noradrenaline in the adrenal glands decreases in hemorrhagic shock and with OHP treatment reaches the normal range.

3) Some correlation between the content of catecholamine in the adrenals and cardiac phosphorylase activity in three groups was seen. The anoxia-induced decrease in cardiac phosphorylase is not due to the lack of catecholamine.

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TABLE 1

THE EFFECT OF HEMORRHAGIC SHOCK AND OHP ON THE PHOSPHORYLASE ACTIVITY OF MYOCARDIUM

CONTROLS			SHOCK			SHOCK +OHP		
A	B	B/Ax 100	A	B	B/A x 100	A	B	B/A x 100
10.3	5.7	55.3	11.6	3.0	25.9	19.8	8.5	45.2
17.6	7.8	44.3	8.7	2.3	26.4	11.9	5.5	47.1
14.1	2.9	20.6	10.0	3.4	34.0	19.3	7.4	38.3
13.9	5.3	38.1	14.7	5.5	37.4	14.2	5.2	36.6
22.0	6.3	28.6	22.3	6.1	26.9	14.6	2.1	14.4
11.3	4.6	40.7	16.6	7.7	42.1	30.2	7.5	24.8
8.2	4.2	35.3	19.8	1.8	9.1			
6.8	2.4	35.3	19.8	4.0	20.2			
18.8	7.9	42.0	23.3	5.7	24.5			
12.1	8.2	67.8	20.9	5.2	24.8			
MEAN		40.8± 2.9			27.1±2.0			34.4±4.0

The assay system for phosphorylase was as follows: 2 per cent glycogen, 0.032 M glucose-1-phosphate, 0.002 M AMP when present, 1.0 ml., total volume, 5 minutes of incubation at 30°C. In column A (units phosphorylase with adenylic acid) and B (units phosphorylase without adenylic acid) the units are expressed by the specific activity. The third Column is the ratio: $\frac{\text{units phosphorylase without adenylate}}{\text{units phosphorylase with adenylate}} \times 100$

Probable error of mean (P.EM.) is expressed as standard deviation x 0.67^{4}.

TABLE 2

CATECHOLAMINE IN ADRENALS

NORMAL			SHOCK			SHOCK + OHP		
Adrenalin	Noradrenalin	Total	Adrenalin	Noradrenalin	Total	Adrenalin	Noradrenalin	Total
237.7	185.0	422.7	35.1	14.1	49.2	163.6	109.8	273.6
304.7	217.0	521.7	151.1	68.2	219.3	187.3	172.7	360.0
155.7	293.7	449.4	109.8	55.2	165.0	561.3	298.3	859.6
203.5	193.3	396.8	156.2	98.6	254.8	194.0	54.0	248.0
188.3	23.8	212.1	484.2	258.2	742.4	20.9	302.0	322.9
138.7	106.8	245.5	86.5	49.3	135.8	221.3	152.7	374.0
342.2	163.1	505.3	165.3	181.0	346.3	443.4	272.8	716.2
403.3	233.8	637.1	42.0	122.3	164.3			
151.4	162.9	314.3	152.6	52.2	204.8			
415.6	390.1	805.9						
593.4	283.0	876.4						
Mean								
285	205	490	153	100	253	256	195	451

Values are expressed as ug. of g. moist tissue.

TABLE 3

RELATIONSHIP BETWEEN CATECHOLAMINE IN ADRENALS AND PHOSPHORYLASE ACTIVITY IN MYOCARDIUM

Group	Catecholamine*			Phosphorylase**
	Adrenaline	Noradrenaline	Total	
Controls	155.7	293.7	449	55.3
	203.5	193.3	397	44.3
	151.4	162.9	314	42.0
	593.4	283.0	876	67.8
	Mean		509	52
Shock	35.1	14.1	49	nil
	165.3	181.0	346	20.2
	86.5	49.3	135	9.1
	109.8	55.2	165	26.9
	151.1	68.2	219	37.4
	Mean		152	19
Shock plus OHP	163.6	109.8	273	45.2
	187.3	172.7	360	47.1
	561.3	298.3	859	38.3
	20.9	302.2	323	14.4
	Mean		454	36

Both determinations in each group were made in the same animals.

* expressed as ug. of g. moist tissue
** expressed as phosphorylase ratio, per cent

APPENDIX D

THE EFFECT OF HYPERBARIC OXYGENATION (OHP) ON THREE FORMS OF SHOCK - TRAUMATIC, HEMORRHAGIC AND SEPTIC

Emil Blair, M.D., George Henning, William G. Esmond, M.D., Safuh Attar, M.D., and R Adams Cowley, M.D.

Shock in whatever form is a continuing menace in the management of disorders within the surgical discipline. Generally hemorrhagic shock is a matter of immediate replacement of lost volumes of blood, and the patient is resuscitated within a very short period of time. On the other hand, if the hypovolemia is permitted to exist for a long period, irreversible processes set in which render resuscitation impossible, regardless of the magnitude of replacement and any supportive therapy. Experimentally, in rapid bleed-out preparations the time factor is in the vicinity of 4 to 4 1/2 hours (14). Shock secondary to bacteremia develops in about 10 to 12% of instances (6). The causative organism is usually of the gram negative coliform type secondary to perforation of a viscus, with an acute peritonitis, or urinary tract complication. Gram positive organisms are the second most frequent and are usually isolated from the peritoneal cavity and the lungs. Until the introduction of antibiotics, the mortality rate was 100% (1). Since that time the mortality rate has been reduced but continues at a high rate of 60 to 70%, despite antibiosis, judicial surgery, and supporative therapy in the form of fluid replacement.

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electrolytes, oxygen, and vasopressors. Traumatic shock even initially presents a complicated problem because of hypovolemia, bacterial contamination, and immunological factors secondary to the injury. All serve to severely tax the homeostasis and host defense mechanisms. Again early therapy, decisive and sure, can be followed by a reasonable degree of recovery. On the other hand, the time factor once more is crucial and beyond a critical point irreversibility develops, much as observed in hemorrhagic and in septic shock.

The precise nature of the pathophysiology is yet to be learned, although many interesting concepts have been advanced. Regardless of the mechanisms involved in the three forms of shock a predominating feature in all three is hypoxia. In general the hypoxia is at the cellular level, the result, as a rule, of critically decreased perfusion. The reduced perfusion may be due to (1) blood loss per se, (2) reduced cardiac output, (3) peripheral vasomotor failure, or (4) a combination of the above. Whatever the underlying mechanism the sum total is a reduction in transport of oxygen below a critical level resulting in the hypoxemia. Since the disorder is primarily a metabolic one because of deficiency of oxygen, a search for other measures in the management of shock has been centered along means of affecting metabolism. One such approach has been hypothermia (3). The view here primarily has been to reduce the metabolic requirements of the cell in the face of the reduced perfusion. The rationale is that reduction of the cell's oxygen needs to a level more commensurate with

what the circulation can deliver may bring the patient back into a more controllable environment. Both experimental and clinical experiences have demonstrated that hypothermia is useful only in bacteremic shock. It can actually be dangerous in hemorrhagic shock since further blood loss during cooling is more poorly tolerated than during normothermia (7). Wartime experiences have suggested effectiveness in traumatic shock, but have not been confirmed (9, 10).

Another approach is to increase the amount of oxygen at the cellular level in the face of deficiency mechanics for oxygen transport. Under normal conditions the partial pressure of oxygen in the alveoli is about 100 mm.Hg. Inhalation of pure oxygen raises this 7 times to approximately 670 mm.Hg. The oxygen tension of arterial blood rises toward the new alveolar level. The hemoglobin, normally about 97% saturated, becomes 100% saturated. In addition more oxygen becomes physically dissolved in the blood. During inhalation of pure oxygen at 1 atmosphere, the total oxygen content of arterial blood is elevated from 20 vol.% to about 22 vol.%, or an increase of 2 vol.%. If the pressure is raised from 1 to 3 atm*, the alveolar oxygen pressure will increase correspondingly and the oxygen tension and content of the arterial blood also will rise. The rise should be approximately 760 mm.Hg. with each increase in atmospheric pressure. At 3 atm. the arterial oxygen tension would be over 2000 mm.Hg., or an increase of about 6 vol.% of arterial oxygen. The resting A-V oxygen difference in a normal person is about 4 to 5 vol.% Therefore, OHP

* atmospheres absolute

at 3 atm should provide enough oxygen for resting metabolism even in the total absence of hemoglobin. Thus, OHP should be able to overcome almost any type of deficiency in oxygen transport.

The characteristic feature in hemorrhagic shock is the reduction in blood oxygen capacity due to loss of whole blood. The increased solution of oxygen into the plasma with OHP would provide the needed amount of oxygen despite the absence of adequate hemoglobin. Similarly, if the hemoglobi within normal limits, but blood flow is reduced because of low output and/or peripheral circulatory failure as in septic shock tissue hypoxia again results. Tissue extracting 6 vol.% theoretically should be kept normally oxygenated at 3 atm with 50% the normal perfusion. In traumatic shock an additional facet aggravating the problem of hypoxia is that of impairment of diffusion of oxygen from the capillaries to the cells. This could, for example, be caused by edema. The elevation of arterial oxygen tension and the increased solubility of oxygen not only in plasma, but also in tissue fluid theoretically should aid in reducing the oxygen deficiency in this type of shock (4).

There have been a number of clinical and experimental reports regarding the efficacy of OHP in various problems created by hypoxia (4,5,12,13). The present report deals with the use of OHP at 3 atm in three forms of shock: traumatic, hemorrhagic, and septic.

METHODS AND MATERIALS

The study was divided into three groups. Group I consisted of traumatic shock produced in rats in a Noble-Collip drum. Two sets of animals were

studied: one a control with drum shock and the other, drum shock treated with OHP. Young adult white male Wistar rats weighing 150-300 gms were used. The animals' legs were taped to a board and drumming was set at a rate of 40 R.P.M. The severity of challenge was varied from 800-840 turns in order to produce severe trauma, but a reasonable survival. Following drumming, the animals were loosened, and the controls allowed to remain in open cages. The second group was transferred to a pressure chamber. This consisted of a top loading autoclave with a pop-off valve in order to insure the pressure in the tank would not exceed 3 atm. Pure oxygen was flowed into the tank at a rate of 1 liter per minute. This, in addition to maintaining the pressure at 3 atm, also provided adequate ventilation in order to avoid accumulation of carbon dioxide within the chamber. The pressure was gradually raised over a period of five minutes to 3 atm and maintained for two hours, after which decompression was effected over a period of fifteen minutes.

(Table 1 here)

The second group (II) consisted of hemorrhagic shock in unanesthetized mongrel dogs, which received premedication with morphine (1.5 mg/kg) one hour prior to bleeding. Following heparinization (2 mg/kg) bleeding was accomplished through a femoral artery cannula. Through another cannula, arterial bleed pressures were measured and blood samples obtained for chemistry. Heart rates were derived from continuous electrocardiographic tracings. The shock preparation employed here was developed at the University of Maryland. It is a modification of the standard Fine hemorrhagic shock dog. The latter

results in irreversible shock in almost 100 per cent of the animals. This proved to be too severe a type of shock for assessment of therapeutic maneuvers. The experimental shock model developed in our Laboratories is an LD_{50} preparation which permits an adequate rate of survival for study of therapy. One group served as the controls and the other similarly bled was treated with OHP. Shock controls were bled to a level of 30 mm. Hg. and maintained at this point for 2 1/2 hours after which the shed blood was reinfused. The dogs in the OHP group after stabilization of the blood pressure at 30 mm. Hg. for 30 minutes were transferred to an OHP chamber and maintained for a period of time equivalent to that of the controls. The pressure in the chamber was raised to 3 atm and maintained for 2 hours after which decompression was achieved in approximately 15 minutes. The animals were then removed from the chamber and reinfused.

Bacteremic shock was induced in chloralosed dogs (Group III) by instillation of a saline suspension of feces. The control breathed room air spontaneously. A second set were treated with OHP under condtions similar to that of the hemorrhagic shock Group. Sampling and pressures were obtained from arterial cannulae.

(Figures 1 and 2 here)

Traumatic Shock: The rats were observed for a 48 hour period after drum shock. The drummed animals presented the typical clinical appearance of severe shock as demonstrated by cyanosis of the noses, paws, and tails. The respirations were shallow and rapid. The rats could be aroused by prodding,

but immediately slipped into a severely obtunded state. The highest mortality rate occurred within 2 hours after drumming. There were 15 rats in the control group of which 9 died, giving a mortality rate of 60% (Table 1). The animals subjected to OHP demonstrated a rapid return of activity. Their color was normal, and their eyes, bright. The rats responded by prodding and defecated and voided freely. Twenty-one rats comprised this group of which only 4 died, a mortality rate of 20%. The difference in mortality was significant to the 5% level.

Hemorrhagic Shock: There were 23 dogs in the hemorrhagic shock control group of which there were only 5 long term survivors, a mortality rate of 83% (Table 1). In the OHP group 14 of 19 survived, a mortality rate of only 26%. This was significant to the 5% level. The arterial blood pressure was kept at 30 mm.Hg. until reinfusion. The hemorrhagic shock state was characterized by tachycardia and hyperpnea. The arterial oxygen content became elevated above preshock level. Carbon dioxide tension was reduced, the pH, acidotic and lactate, elevated. In OHP treated group the arterial blood pressure began to rise after the first hour of compression and at the end of 2 hours averaged 90 mm.Hg. for the group. In the chamber the compensatory tachycardia was replaced with a slower rate. (Figure 1). The hypocarbia and acidosis were similar as in the control shock dogs and the lactate elevation of greater magnitude (Figure 2 and 5). Upon decompression and reinfusion, the heart rate at first accelerated and then declined toward pre-shock level. The lactate decreased and the pH rose.

(Figures 3 and 4 here)

Septic Shock: There were 10 dogs in each group. Within six hours of innoculation, 9 of the 10 dogs were dead in the control group and 8 of 10 in the bacteremic OHP group. The biochemical picture presented a severe metabolic acidosis manifested by reduced pH, elevated lactate, and decreased in buffer base. The A-V oxygen difference widened considerably. The arterial blood pressure dropped to shock levels and was accompanied by a compensatory tachycardia and hyperpnea (Figure 3). The OHP group demonstrated an initial retardation of the adverse changes for the first hour, but thereafter a rapid deterioration set in until the biochemical picture became worse than that of the control bacteremic shock group (Figure 4 and 5). Again the compensatory tachycardia was diminished in the OHP group.

(Figure 5 here)

DISCUSSION

Traumatic and hemorrhagic shock demonstrated significant improvement in survival with OHP treatment, while septic shock failed to show any. Since hypoxia is the predominant problem in all three syndromes, and OHP provides a tremendous delivery of oxygen, the reason for the difference in response is not entirely clear. It is apparent that merely increasing the amount of oxygen available at the cell is not sufficient to improve the pathologic picture in bacteremic shock. The most suggestive reason for the differences in response to OHP is related to endotoxemia (8). Regardless of the availability of oxygen it would appear that the endotoxins had overwhelmed the host defense mechanisms and also handicapped the cell's ability to metabolize. Oxygen at high pressure

inhibits bacterial growth as demonstrated by in vitro studies (2). The failure to do so in these studies may be due to the fact that the high pressure and oxygen conditions within the OHP tank were not transmitted to the animal and hence to the site of bacterial growth; i.e., the peritoneal cavity. Here the content of oxygen was merely elevated, probably allowing a more luxuriant growth of bacteria and thus an increase in elaboration of endotoxins. This is suggested by the fact that the biochemical picture of the OHP bacteremic animals was much worse than that of the bacteremic control dogs. Whatever the cause, OHP failed completely to alter the course of events in bacteremic shock while there was significant improvement in the other two forms of shock.

This raises the question with regard to a proposed unitarian theory for the irreversibility of shock (11). This proposal suggested that endotoxemia is the basic cause for irreversibility in shock regardless of the initial type of shock which may have developed. If this were true, since endotoxins are the agents responsible for pathologic changes in bacteremic shock, OHP should not have improved conditions in the other two forms of shock. These studies would suggest that the three types of shock do not have a common etiology, nor necessarily a common mechanism with regard to the development of irreversibility leading to death.

SUMMARY

Three forms of shock were induced experimentally in order to evaluate OHP in the management of shock. Traumatic shock was induced in rats and OHP resulted in a significant improvement of survival rate. Hemorrhagic shock was induced in dogs and again the survival rate significantly improved with OHP.

Bacteremic shock in dogs, however, failed completely to respond to OHP therapy. The reasons for this are not yet entirely clear. Apparently merely increasing the oxygen available at the cell is not sufficient to even delay the onset of irreversibility. This suggests that the pattern of the pathophysiologic changes in bacteremic shock may be different from that in hemorrhagic and traumatic shock, at least insofar as the role of oxygen may be concerned.

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LEGENDS

Figure 1. In hemorrhagic shock the heart rate rises with the fall in arterial blood pressure. The tachycardia continues until just before death. OHP at 3 atm inhibits the tachycardia and causes a rise in the arterial blood pressure. The pre-hemorrhage pressure, however, is not reached until the shed blood is reinfused. BS = Before shock; HS = Hemorrhagic Shock; RI = Reinfusion

Figure 2. The arterial oxygen content rises in the control shock animals as the result of compensatory hyperpnea. With OHP this increase is much greater. Arterial pCO_2 in the controls is unchanged until reinfusion and irreversibility. Exposure to OHP cause an increase with a fall after decompression. The acidosis is not as severe and is reversible with OHP treatment.
BS = Before Shock; HS = Hemorrhagic Shock; RI = Reinfusion.

Figure 3. In septic shock treated with OHP the compensatory tachycardia is inhibited while the hypotension is progressive as in the shock controls.
BS = Before Shock; SS = Septic Shock.

Figure 4. Arterial oxygen content rises in the control shock, but is much greater with OHP. The pCO_2 tends to increase in the OHP chamber. The acidosis is equally severe in both groups.
BS = Before Shock; SS = Septic Shock.

Figure 5. Lactic acid in the hemorrhagic shock (HS) controls and in the OHP group rises progressively, with a fall in the latter after decompression. In the septic shock (SS) OHP group the elevation in lactic acid was significantly greater than in the shock controls.

TABLE 1. OHP EFFECT ON SURVIVAL RATES IN SHOCK

TYPE OF SHOCK	CONTROLS No.	Survived	%	OHP No.	Survived	%
Traumatic	15	6	40	21	17	81
Hemorrhagic	20	3	17	18	14	74
Septic	10	0	0	10	0	0

EFFECT OF HYPERBARIC OXYGEN (OHP) ON GRAM NEGATIVE BACILLI

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This study was designed to note the effect of OHP on Gm. neg. bacteria using matched in-vivo and in-vitre studies. In-vitro studies: measured innocula of bacteria were subjected to varying pressures of O_2 at 37°C. O_2 tension at 2 and 3 atm. resulted in a bacteriostatic effect (E. coli). O_2 tension up to 1 atm. increased the bacterial growth rate while all pressures of air up to 3 atm. showed no change over normal pressure. In-vivo studies: dogs were infected peritoneally with a measured fecal suspension. Studies were done at varying pressures of O_2 by taking periodic quantitative blood cultures. The dogs with a closed abdomen at 3 atm. O_2 showed a peritoneal O_2 tension of no more than 80 mm. Mg. whereas the open abdomen gave the full intraperitoneal effect of O_2 at 3 atm. Infected dogs at 3 atm. of O_2 with an open abdomen showed an initial septicemia at 1 hr. The blood was relatively cleared after 2 1/2 - 3 1/2 hrs. After this clearing, the septicemia recurred, increased, and resulted in the death of the dog in 8 $\pm$ hrs. Those dogs with the abdomen closed showed an increasing septicemia from the beginning and died after 5 $\pm$ hrs. as did the dogs treated with air and an open abdomen at 3 atm. pressure. If oxygen is allowed to reach the bacteria with an established pressure of 2 - 3 atm., the bacteriostatic effect may be realized and the dog's defenses prevail until

an overwhelming tissue invasion takes place. Clinical treatment of fecal peritonitis with OHP will require ventilation of the cavity involved plus the traditional supportive measures and surgery.

THE EFFECT OF HYPERBARIC OXYGENATION (OHP) ON BACTEREMIC SHOCK

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Bacteremic surgical shock most often is caused by gram negative coliform microorganisms.[1,2] However, any microorganism which produces septicemia can be responsible for this too frequently lethal syndrome. The incidence of shock from bacteremia is about 10-12%.[3] A positive blood culture is diagnostic. However, shock occurs with a severe peritoneal or pulmonary sepsis and negative blood cultures. Despite the intensive use of appropriate antibiosis, blood replacement, vasopressors, judicious surgery and supportive measures, the mortality rate from bacteremic shock continues to be extraordinarily high -- approximately 60-70%. Because of the difficulty in adequately resuscitating the patient in shock, other measures for the management of this devastating problem have been pursued, including hypothermia.[4] While some promise of success has developed, the tremendous morbidity and the continued high mortality rate has not been altered appreciably.

Although the pathophysiology of bacteremic shock has not been fully elucidated, the net result is a severe hypoxemia.[2,5] A principal problem appears to be one of the maintenance of an adequate oxygen tension at the cellular

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level. Hyperbaric oxygenation (OHP) has been demonstrated to be of striking value in a number of clinical problems and experimental designs in which hypoxia is a dominant feature.[6,7] The rationale in OHP is simply to dissolve more oxygen into the plasma, so that more of it will be available to the cell.

METHODS AND MATERIALS

Randomized mongrel dogs under chloralose anesthesia were subjected to bacteremic shock of the gram negative coliform variety by the instillation of a saline suspension of feces into the peritoneal cavity. Two groups of animals were studied. One consisted of a control shock group (I) and the other, shock treated with OHP at 3 atm* (II). Under fluoroscopic guidance a #8 Cournand catheter was placed into the pulmonary artery via a jugular vein for mixed venous sampling. Arterial blood was obtained from a cannulated femoral artery. Heart rates were obtained from ECG traces. The arterial blood pressure was recorded through the femoral cannula using a Statham transducer and a carrier type amplifier fitted with a direct writer. Mean pressures were obtained by electronic integration. Blood samples were obtained prior to instillation of feces, after onset of bacteremic shock and at intervals to death. Because of difficulties in obtaining blood through the OHP chamber, sampling in this group was limited to one hour and just prior to death. The blood was analyzed for oxygen and carbon dioxide content, for lactates and p^H. Bicarbonate and pCO_2 were derived from the homogram of McLean.**

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* atmospheres absolute

** Physiological Reviews, 1938, 18:495

The OHP chamber consisted of a modified autoclave tested to withstand 6 atm. Oxygen, supplied from a standard pressure cylinder, was precooled to 10°C during flow into the chamber. Cooling the gas was essential to prevent overheating in the chamber, the temperatures of which was maintained at 25°C. A spring loaded pop-off valve was designed to prevent the chamber pressure from rising above 31 lbs/in^2 in the event of malfunction of the cylinder reducing valve. Compression to 3 atmospheres was achieved in 15 minutes. Excess carbon dioxide was washed out through an escape vent.

RESULTS

The death rate of both groups appear in Table 1. Within 6 hours of contamination 9 of 10 of the controls and 8 of 10 of the OHP group had succumbed. In another 2 hours, the death rate was 100% for both groups. The picture of bacteremic shock was characterized by a sudden drop in arterial blood pressure with tachycardia and tachypnea. Hemodynamic data are illustrated in Figure 1. A singular feature was marked widening of the A-V oxygen difference, often three or four times the baseline value. The hematocrit rose significantly. A metabolic acidosis developed (Table 2) manifested by low pH, reduced buffer base, low pCO_2, and elevated lactates. Blood cultures were uniformly positive at this time.

Following onset of bacteremic shock the control group (I) demonstrated progressive increase in A-V oxygen, heart rate, hematocrit and decline in arterial blood pressure. The metabolic acidosis worsened until death. However, oxygen content was persistently in normal range. In Group II after one hour

of OHP the hemodynamic changes were similar to the controls. However, as OHP was continued the heart rate slowed and dropped below preinnoculation levels. The A-V oxygen in the first hour of OHP did not continue to increase as the controls. However, after this time the biochemical picture rapidly deteriorated and actually became worse than the controls. Arterial oxygen content throughout was higher than that in the control shock animals.

DISCUSSION

The physiologic picture of bacteremic shock in these studies are similar to that of other investigators.[9,10] The hypoxia results from reduced tissue perfusion (evidenced by widening A-V oxygen), which is believed due to (1) decreased cardiac output and (2) peripheral vasomotor failure.[9] The intracellular aerobic inanition results in incomplete oxidation of pyruvates and leads to accumulation of fixed acids; hence the metabolic acidosis. Carbon dioxide tension is low due to the compensatory hyperpnea from "air hunger", which also maintains alveolar pO_2, evidenced by normal arterial oxygen content. The accelerated heart rate is an attempt to maintain output but this soon becomes ineffectual. The deficiency in transport of oxygen is further aggravated by the loss of plasma volume as suggested by the elevated hematocrit. In this type of experimental model, a significant portion of this loss is into the peritoneal cavity. The accelerating and combined attack of endotoxins and hypoxia lead to a dissolution of the compensatory forces and to a complete rout of the host-defense system. Death ensues.

In view of the known increased availability of oxygen under OHP conditions, it seemed rather remarkable that the death rate in both groups was almost

identical. Normally the alveolar partial pressure of oxygen can be increased from 100 to 670 mm. Hg. by breathing pure oxygen instead of air. The hemoglobin becomes fully saturated and more oxygen goes into the plasma. Arterial oxygen content is increased by 2 volumes per cent. When the pressure of oxygen is raised to 3 atm, the arterial content is further elevated to 6 volumes per cent, (P_{AO_2} =2000+ mm.Hg.). The increase in oxygen in solution is not confined to blood plasma, but also the whole body, including interstitial fluid, as well [illegible] the solubility of oxygen in body fluids increases by 2.3 volumes per cent for every increase of one atm.[8] The body is therefore capable of storing oxygen. The normal A-V oxygen difference is between 4 and 5 volumes per cent. This means that at rest this amount of oxygen is sufficient for metabolism. This could be provided easily by OHP at 3 atm. even, in the complete absence of hemoglobin.[7] Thus, at least theoretically, almost all oxygen transport deficits should be overcome.

The changes with OHP were inhibited temporarily as evidenced by the arrest in A-V oxygen widening in the first hour. However, despite the available oxygen, the subsequent course was identical to that of the controls. This would suggest that some mechanism was interfering with the metabolism of oxygen by the cell. The most obvious cause would be the effect of endotoxins elaborated by the coliform bacteria. The biochemical changes in the OHP group after the first hour became more severe than the controls. The reason for this is not clear. The possibility of oxygen toxicity does exist. This is suggested in the failure to maintain the compensatory tachycardia, which was maintained

or increased in the control group. However, in another study we demonstrated OHP at 3 atm to be of significant benefit in hemorrhagic shock.[6] While hypoxemia is a significant problem in both hemorrhagic and bacteremic shock, the mere increase in oxygen to the cell is not sufficient to prevent irreversibility in the latter problem.

The crux of the difficulty probably lies with the effect of the toxins of microörganisms upon homeostasis including the host defense mechanisms. Gram negative coliform bacteria are essentially aërobic and grow abundantly in atmospheric air. In these experiments exposure of the septic animal in the OHP chamber leads to a higher concentration of oxygen available not only to the host, but also to the bacteria. This increased oxygen very likely permits more luxuriant growth of the offending microörganisms, and hence an increased elaboration of endotoxins. This increased release of endotoxin can overcome whatever difference may have been gained by the increased availability of oxygen to the host.

It has been demonstrated through <u>in vitro</u> studies that OHP inhibits bacterial growth, while air at higher pressures does not.[11] The important factor is high oxygen tension with the increased pressure secondary. In the intact animal the total OHP effect is not direct but transmitted through the tissue and, therefore, is likely reduced. Current studies in our Laboratories are being directed to this problem. The abdomen is opened to permit the development of a higher pressure into the peritoneal space prior to exposure to OHP. Through this maneuver the pressure in the peritoneal cavity soon becomes equilibrated with

the pressure in the chamber -- essentially 3 atm. Under these circumstances preliminary studies indicate the survival period is significantly prolonged over that of the control animals, or in the OHP group with the abdomen intact. However, it is important to point out that all of these animals have succumbed also. These studies suggest OHP may not be of value in the treatment of bacteremic shock per se. But emergency surgery in the face of generalized peritonitis in an OHP chamber may prove valuable.

Another serious problem which requires further elaboration, of course, is the effect of OHP itself on the normal physiology. Oxygen toxicity is characterized by disorders of the brain and of the lungs primarily.[12] Convulsions may occur and pulmonary edema has been reported in animals as well as in man. However, these effects occur as a rule only in prolonged exposure, or at pressures higher than 3 atm.

Bradycardia is a characteristic feature of OHP.[12] Between 3 and 4 atm acid-base balance and blood chemistries were not found to be greatly altered in experimental animals.[13,14] According to Behnke OHP at 3 atm for 3 hours is safe for man.[12]

SUMMARY

Gram negative coliform bacteremic shock was induced in dogs by instillation of a saline suspension of feces into the peritoneal cavity. The pathophysiologic picture is similar to the clinical syndrome and is characterized by hypotension, tachycardia, wide A-V oxygen difference and metabolic acidosis. One group of 10 dogs served as controls and another equal number were treated with OHP at 3 atm. The mortality was 100% in both groups with no difference

in survival time. The increased available oxygen apparently failed to alter the outcome. A suggested series of events seems to be a paradoxical effect, with enhancement of bacterial growth and endotoxin production, which overcomes any possible benefit of OHP to the host. Inhibition of bacterial growth may not be obtained in the intact animal due to loss of maximum OHP effect.

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Table 1, Cumulative Death Rates

	Bacteremic Shock			Bacteremic Shock + OHP		
Hours*	No.	%	Total %	No.	%	Total %
3	1	10	10	2	20	20
4	2	20	30	0	0	20
5	1	10	40	1	10	30
6	5	50	90	5	50	80
7	0	0	90	1	10	90
8	1	10	100	1	10	100

* From time on innoculation.

Table 2, Biochemical Data

		pH		pCO_2 mm. Hg.		HCO_3 mEq/L		Lactate mg %		Art. O_2 Vol.%	
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mear	S.E.
Before Infection	I.	7.40	.10	35.7	3.3	29.4	1.3	27.7	5.2	15.8	1.1
	II.	7.35	.01	39.2	2.1	20.3	.6	27.6	5.1	15.4	.6
Bacteremia	I.	7.26	.11	30.5	3.1	13.7	1.2	40.2	6.9	20.0	1.1
Control	I.	7.16	.15	19.8	1.7	11.3	1.5	47.5	12.0	19.8	1.7
OHP (1 hour)	II.	7.22	.07	29.8*	4.2	10.0	.8	65.0*	12.3	23.3	1.8
Control	I.	7.00	.11	25.0	2.4	9.5	4.3	63.4	14.6	20.1	1.4
OHP	II.	7.05	.10	34.4*	5.7	10.6	2.9	102.8*	35.6	23.9	1.4

*Significant to the 5% level compared with Group I.

THE EFFECT OF HYPERBARIC OXYGENATION (OHP) ON BACTEREMIC SHOCK

(Abstract)

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Assuming normal utilization, oxygenation of tissue is a function of the amount of oxygen needed and the amount available. Bacteremic shock is characterized by tissue oxygen deficit due to reduced perfusion. Oxygen at 3 atmospheres absolute elevates arterial oxygen content from about 20 to 26 volumes per cent. The increase is principally in the form of physically dissolved oxygen. Therefore, oxygen availability to the tissue should be enhanced and the hypoxia relieved.

Gram negative bacteremic shock was induced in 2 groups randomized chloralozed dogs by intraperitoneal instillation of feces. The shock state was characterized by: hyperpyrexia, hyporeflexia, hypotension, tachycardia, hyperpnea, reduced cardiac output, widened atriovenous (A-V) oxygen difference, normal or elevated oxygen consumption, normal arterial oxygen saturation, low or normal carbon dioxide content and metabolic acidosis.

All of the animals in the control group were dead within 6 hours. The OHP treated group showed an initial improvement, manifested by some decrease in A-V oxygen difference. However, these dogs expired in about the same period of time.

The reason for failure of OHP to improve on the mortality is not known. Several factors may be responsible. (1) Oxygen toxicity is a

potential hazard and may have neutralized any initial benefits. However, we have demonstrated OHP treatment of hemorrhagic shock to be highly effective. Elevated temperatures increase oxygen toxicity, but the chamber temperature was controlled. (2) Aerobic bacteria flourish in a higher oxygen environment. OHP may further enhance growth and toxin formation. (3) OHP may have a deleterious effect on the reticuloendothelial system.

THE UTILIZATION OF HYPERBARIC OXYGENATION IN HEMORRHAGIC SHOCK IN DOGS

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The beneficial effects of administration of high concentration of oxygen in various states of shock were recognized as early as 1939. Boothby, Mayo and Lovelace[2] advocated the inhalation of 100 per cent oxygen in shock associated with trauma, surgical collapse, pulmonary embolism, septicemia following peritonitis, and anerobic infections.

In 1940, Wood, Mason and Blalock[7] produced hemorrhagic shock in inhalation of 100 per cent oxygen for only fifteen minutes increased the arterial and venous blood oxygen content, and the blood pressure rose 6 mm of mercury. The effect of more prolonged oxygen therapy was not reported. In 1941. Schenedorf and Orr[6] demonstrated that inhalation of 100 per cent oxygen enables the treated dogs to tolerate 15 per cent greater blood loss, and 17 per cent increase in the survival time over the control dogs. In 1962, Manger and Hahas[5] demonstrated an increase in the survival rate of shocked dogs from 10 per cent breathing room air to 70 per cent by the combination of pH control and inhalation of 100 per cent oxygen.

In 1960[3], we produced irreversible hemorrhagic shock in 45 dogs by means of the Fine technique, of these dogs, 25 were used as controls, while the remaining 20 were perfused with the pump oxygenator at the end of reinfusion

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when the blood pressure dropped to 80 mm Hg., their perfusion being maintained at this pressure for one hour. The average survival time of the perfusion group was increased almost three times that of the control (from 2 hours 24 minutes to 6 hours 52 minutes). A concomittent improvement was observed in the blood chemistries studied. This was attributed to the improved oxygenation of hypoxia tissues, although the irreversible trend could not be altered.

In order to continue our studies on the effect of improved tissue oxygenation on the survival of experimental animals in hemorrhagic shock, a modification of the Fine preparation was developed that proved more suitable for assessment of various therapeutic measures, including hyperbaric oxygenation (OHP).

MATERIALS AND METHODS

Five groups of animals were studied:

Group A - Control - Hemorrhagic Shock - Room Air

Group B - Hemorrhagic Shock - High pressure oxygen (OHP)

Group C - Control - OHP

Group D - Hemorrhagic Shock - Room Air - THAM

Group E - Hemorrhagic Shock - OHP - THAM

The control shock group breathing room air (Group A) comprised 35 dogs, averaging 9.15 Kilograms in weight. One hour prior to induction of bleeding, each animal was given morphine sulfate (1.5 mgm per kilogram of body weight) by the intramuscular route. All dogs were heparinized using 2 mgm per kilogram of body weight of aqueous heparin intravenously. Both femoral arteries were cannulated using one per cent procaine anesthesia

locally; one was connected with the elevated reservoir, the other with a mercury manometer. Bleeding was accomplished via the femoral artery cannula; and the blood entered an elevated heparinized reservoir, the height of which was adjusted to 40 cm above the auricular level, the blood pressure being maintained at 30 mm Hg. After the maximum bleed-out was reached (46.0 cc per Kg body weight), there was a spontaneous uptake averaging 26.7 per cent of the shed blood. A mea.. arterial pressure of 30 mm Hg. was maintained for 2-1/2 hours after which the shed blood was reinfused slowly. The dogs were observed for several hours before being returned to the cages.

Group B, which was treated with high pressure oxygen, consisted of 25 dogs averaging 12.6 kilograms in weight. Shock was produced by hemorrhage in a fashion similar to Group A. The average bleed-out volume was 49.7 cc per kilogram of body weight. After the dogs had stabilized at an arterial pressure of 30 mm Hg. for 30 minutes, they were disconnected from the reservoir and introduced into the oxygen chamber, where a pressure of 3 atmospheres absolute was obtained using oxygen as a compressor medium. Compression and decompression were gradual, each requiring 15 minutes, the total compression period being equivalent to the duration of shock in Group A.

Group C consisting of 13 dogs comprised the control group subjected to high pressure oxygen under morphine narcosis at 3 atmospheres for 2-1/2 hours.

Group D comprised six dogs that were subjected to the same hemorrhagic shock preparation as Group A. However, the arterial pH was monitored and after reinfusion of the shed blood, it was corrected using a buffer solution containing 0.2 M Tris (hydroxymethyl) aminomethane and 0.05 M $NaHCO_3$.

Group E comprised of five dogs which were subjected to the shock preparation used in Group A. The metabolic acidosis was corrected with THAM buffer solution after reinfusion of the shed blood, and then the animals were treated with high oxygen pressure at 3 atmospheres for 2-1/2 hours. The average bleed-out volume in this group was 49.7 cc per kilogram of body weight.

Arterial, venous, and mixed venous bleed samples were drawn before hemorrhage, with the dog in shock prior to introduction into the chamber, immediately after decompression, and one hour after reinfusion. The samples were examined for pH, CO_2 content, hematocrit, lactic acid, and oxygen saturation. PCO_2 and plasma bicarbonate were calculated from the Singer and Hastings nomogram. In some dogs, tissue oxygen tensions were determined and recorded continuously by way of the Clark needle electrode inserted in either muscle or liver. All dogs that died within 96 hours after the procedure were autopsied. Gross and histologic sections were obtained. Those surviving longer were sacrificed at various intervals.

THE OXYGEN CHAMBER

The pressure chamber used in these experiments was described elsewhere in details[1]. It consisted of a converted autoclave, 17 inches in diameter and 4 feet in length. Accumulation of carbon dioxide was prevented by constant flushing of oxygen at the rate of 10 L per minute. Arterial blood pressure, rectal temperature, electrocardiogram and oxygen tensions were monitored through appropriate cables leading from the chamber.

PHYSIOLOGIC DATA

Body temperature was observed to drop an average of 2.5°C in the hemorrhagic shock group breathing room air (Group A). In the OHP group, there was an average increase of 1°C although marked hyperthermia (41°C) was seen

in hyperactive and convulsing animal exposed to OHP. Marked tachycardia was observed in Group A, which increased during compression in Group B, whereas bradycardia was noted to occur upon compression of normal dogs to OHP (Group C). This trend was reversed by decompression and reinfusion of the shed blood.

In Group B, the arterial blood pressure rose an average of 25 mm Hg. immediately upon compression, and remained elevated until reinfusion. This phenomenon was not observed in the dogs which did not survive; on the contrary, hypotension below 30 mm Hg. was a constant feature.

The electrocardiographic changes seen after bleeding included a change in the configuration of the P waves, with a marked decrease in the amplitude of the QRS complex. During exposure to OHP, a depression of the ST segment was constantly observed, the T wave becoming tall and sharp. Atrial fibrillation and atrioventricular dissociation was occasionally seen. Three dogs developed ventricular tachycardia followed by ventricular fibrillation and death one hour after compression at 3 atmospheres absolute. All changes in the various complexes of the electrocardiogram reverted toward normal after decompression and reinfusion.

An average decrease in respiratory rate amounting to 50 per cent of the base line was noted in Group B during the compression period. However, hyperpnea was noted upon decompression and reinfusion amounting in some instances to 300 respirations per minute, which required few hours before returning to baseline levels.

The muscle oxygen tension was found to drop significantly from 36 to 10 mm Hg. during shock. In the oxygen chamber, the tension rose to about 100

mm Hg. and then stabilized at 50 mm Hg. to drop back to normal after decompression. The oxygen tension of the liver showed a lesser drop in shock. There was an immediate rise to 100 mm Hg. as soon as the chamber pressurized, but it then stabilized at 40 mm Hg.

BIOCHEMICAL CHANGES

The biochemical changes seen in the hemorrhagic shock group (Group A) were those of metabolic acidosis, secondary to hypoxia of the tissues, and anerobic metabolism. There was a marked fall in pH, arterial and venous oxygen saturation, a decrease in bicarbonate, and a marked increase in lactic acid. In Group B and C the oxygen saturation showed a definite increase in the arterial blood. The oxygen saturation of the mixed venous blood was not measured in the chamber; however, it was moderately increased immediately after decompression. The arterial PCO_2 was observed to drop in both groups after exposure to OHP. The mixed venous PCO_2 increased at the end of decompression in the shock group treated with OHP, whereas an opposite change was seen in the control group (Group C). The lactic acid showed a marked increase in Group B and C during exposure to OHP, with reversal to normal values after decompression and reinfusion.

RESULTS

From our experience with hemorrhagic shock, it became apparent that most of the deaths occur within the first 24 hours of termination of the experiment. Very rarely some dogs succumb within 24 to 48 hours. Therefore we considered dogs surviving 48 hours or longer as long term survivors. In the control group breathing room air (Group A) five dogs died of cardiorespiratory arrest early in the

experiment before reinfusion of the shed blood. These were considered incomplete experiments and were excluded. Of the remaining 30 dogs, 23 died within 24 hours, and 2 died 2 days after the procedure. Only 5 were long term survivors which gives a survival rate of 17 per cent.

In Group B which was shocked and treated with OHP, 6 dogs were excluded as incomplete experiments, 5 died within 48 hours, 2 within 96 hours and 12 were long survivors. We believe that with better care the animals that died within 96 hours could have been saved. The survival rate can thus be considered significantly increased from 17 to 74 per cent.

In the OHP control group (Group C) five animals were excluded for technical reasons (fatal hemorrhage, etc) of the remaining 8, 6 survived and were ultimately sacrificed. All animals that were treated with THAM died within 5 to 7 hours after reinfusion.

Finally in Group E only 2 dogs were long term survivors.

DISCUSSION

Since the oxygen requirements of the tissues in shock remain the same, but the oxygen delivery and utilization are impaired, an oxygen debt develops averaging from 60-100 cc O_2 per kilogram of body weight. When this debt exceeds 120-130 cc O_2 per kilogram, irreversibility develops. At one atmosphere pressure about 20 cubic centimeters of oxygen are carried combined with hemoglobin in each 100 cubic centimeters of blood, and 0.3 cubic centimeters of oxygen is carried in simple physical solution in the plasma. At three atmospheres, the physically dissolved oxygen is increased to 6.6 cubic centimeters and can provide all the oxygen requirements for cellular respiration. Therefore the tissues can survive despite the lack of oxygen carried through the

red blood cells. This is amply demonstrated not only in the survival rate of the treated animals, but also in the improvement of the physiological and biochemical determinations reflecting the salutary effect of OHP at the intracellular level.

Despite the beneficial effect of OHP, this method of therapy carries the risk of oxygen poisoning. The fundamental disturbance in the latter is the irreversible oxidation of respiratory enzymes, especially those with sulfhydril group. In the experiments reported above, only 2 dogs from Group B (shock and OHP) demonstrated oxygen toxicity. One convulsed during compression and died of respiratory failure upon decompression; the second developed paralysis of the hind legs after compression, but survived. For the control OHP group (Group C), oxygen toxicity occurred more frequently. Three dogs developed convulsions, hyperpnea and hyperthermia, and at autopsy demonstrated extensive hemorrhagic lesions in the heart and lungs.

The rationale behind the use of THAM in Group D and E was not only to correct acidosis secondary to hemorrhagic shock, but also to protect the animals against oxygen toxicity. Bean demonstrated the efficacy of THAM in buffering tissue PCO_2, delaying the onset of the oxygen seizure, and decreasing their incidence and severity. Oxygen toxicity was not encountered in Group E although the series is too small to be significant.

SUMMARY AND CONCLUSION

High pressure oxygen at three atmospheres has been found to alter the irreversible stage in hemorrhagic shock and increase the survival rate from 17 per cent in the control group to 74 per cent.

The beneficial results are attributed to the increased amount of physically dissolved oxygen which maintains the oxidative processes at the cellular level despite reduced oxygen transport by hemoglobin.

EXPERIMENTAL ASPECTS OF THE USE OF HYPERBARIC OXYGEN IN HEMORRHAGIC SHOCK

Safuh Attar, M.D., William G. Esmond, M.D., R. Adams Cowley, M.D.

Oxygen inhalation at 1 atmosphere pressure was demonstrated to be beneficial in states of shock by Boothby[2], Wood and Blalock[7], Schenedorf and Orr[6], and recently by Manger and Nahas[5]. Since the oxygen requirements of the tissues in shock remain the same but the oxygen delivery and utilization are impaired, an oxygen debt develops averaging from 60 to 100 cc O_2/kg body weight. When oxygen debt exceeds 120-130 cc O_2/kg irreversibility develops[4]. At 1 atmosphere pressure about 20 cubic centimeters of oxygen are carried combined with hemoglobin in each 100 cubic centimeters of blood and 0.3 cubic centimeter of oxygen is carried in simple physical solution in the plasma. At 3 atmospheres the physically dissolved oxygen is increased to 6.6 cc, and can provide all the oxygen requirements for cellular respiration. Therefore, the tissues can survive, despite the lack of oxygen carried through the red blood cells, or in other words, the loss of blood in hemorrhagic shock can be compensated for by physically dissolved oxygen. To test this hypothesis, hyperbaric oxygenation was utilized in the treatment of hemorrhagic shock, studying its effects on the biochemical changes as well as survival rate.

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C5)

METHODS

Sixty mongrel dogs averaging 11 kg were used in this study. Thirty-five dogs (Group A) comprised the control shock group, breathing air at atmospheric pressure. Twenty-five dogs (Group B) were subjected to hemorrhagic shock as in Group A and treated with high pressure oxygen (OHP). Morphine sulfate (1.5 mgm/kg) was given intramuscularly 1 hour prior to induction of shock. All dogs were heparinized using 2 mgm/kg body weight of aqueous heparin intravenously. Both groups were bled to a mean arterial pressure of 30 mm Hg. This pressure was stabilized within 30 minutes while breathing room air. The shed blood averaged 46 cc/kg in Group A and 49.7 cc/kg in Group B. Group A remained in shock for 2 1/2 hours after which the shed blood was reinfused intravenously. Dogs in Group B were disconnected from the blood reservoir and introduced into an oxygen chamber where a pressure of 3 atmospheres absolute was obtained using oxygen as a compression medium. Compression and decompression were gradual, each requiring 15 minutes, the total compression period being equivalent to the duration of shock in Group A.

In the majority of these pressurized experiments no facility was available to reinfuse blood into the shocked dogs when their blood pressure dropped below 30 mm Hg. In later experiments, it was possible to reinfuse blood from a small chamber pressurized to 3 atmospheres. In a few dogs samples of mixed venous blood were obtained via a cardiac catheter introduced through the jugular vein.

Arterial, venous and mixed venous blood samples were drawn before hemorrhage, in shock prior to compression, immediately after decompression, and 1 hour after reinfusion. Blood was examined for pH, CO_2 content, hematocrit, lactate, and oxygen saturation. PCO_2 and plasma bicarbonate were calculated from the Singer-Hastings nomogram.

THE OXYGEN CHAMBER[3] (Fig. 1)

The chamber used in these experiments consists of a modified autoclave 17" diameter and 4' in length. A plexiglas window in the autoclave door permitted monitoring of the arterial blood pressure from a mercury manometer. Oxygen is provided from a standard pressure cylinder through a reducing valve that can supply gas at any desired pressure. The chamber is also provided with an exit vent pipe which contains a spring loaded pop-off valve designed to prevent pressure rise above 30 pounds/square inch.

RESULTS

Body temperature was observed to increase an average of 1° C in the pressurized group, compared to a drop of 2.5° C in the hemorrhagic shock group. Pulse rate increased rapidly in both groups during the hypotensive period, although bradycardia is the normal response to compression at high atmospheric pressures. In Group B the blood pressure rose an average of 25 mm Hg immediately upon compression and remained elevated till reinfusion. This phenomenon was not observed in the dogs which did not survive; on the contrary hypotension

below 30 mm Hg was a constant feature. An average decrease in respiratory rate amounting to 50% of the baseline was noted in Group B during the compression period. However, hyperpnea was noted upon decompression and reinfusion amounting in some instances to 300 respirations per minute, which required few hours before returning to baseline levels.

The biochemical changes are summarized in (Fig. 2). Although a significant drop in pH was seen in both groups, the arterial pH in the OHP group improved at the end of compression, and all values returned to normal after decompression and reinfusion. The oxygen saturation showed a definite increase in both arterial and mixed venous blood which would have been significantly higher had the samples been obtained during the compression period, rather than immediately after decompression. These results contrast with Group A breathing room air during the shock which had lowering of O_2 saturation and a marked increase in A-V oxygen difference. The bicarbonate levels dropped in both groups during shock and OHP. The arterial PCO_2 was observed to drop after exposure to OHP, although the mixed venous blood showed a marked increase. The lactic acid was markedly increased in both groups with reversal to normal values after decompression.

Figure 3 summarizes the survival rate in both groups. In the control group, 5 dogs were incomplete experiments, 23 died within 24 hours, and 2 died 2 days after the procedure. Only 5 were long term survivors giving a survival rate of 17%. In the

high pressure oxygen group 14 survived 48 hours after the procedure giving a survival rate of 74%.

DISCUSSION

Schenedorf and Orr demonstrated that inhalation of 100% oxygen enabled the treated dogs to tolerate a 15% greater blood loss, and 17% increase in the survival time over the control dogs. Manger et al demonstrated an increase in the survival rate of shocked dogs from 10% breathing room air to 70%, by the combination of pH control and inhalation of 100% oxygen. Our experiments demonstrate clearly the beneficial effects of high pressure oxygen on the irreversibility of hemorrhagic shock. This effect must be sought at the cellular level. The main role of oxygen is an electron acceptor. In the cellular oxidative processes approximately 30 substrate specific dehydrogenases start the chain at the reducing end near the substrate. They are followed by flavoproteins and cyotchromes. The terminal oxidase transmits electrons to oxygen. The resulting oxygen can react with hydrogen ions to form water. Through these oxidative processes energy is liberated and utilized to convert adenosine diphophaste to adenosine triphosphate.

Despite the beneficial effects of high pressure oxygen, this method of therapy carries the risk of oxygen posioning. The fundamental disturbance in the latter is the irreversible oxidation of respiratory enzymes, especially those with sulfhydril groups. In the experiments reported above, only 2 dogs treated with OHP demonstrated oxygen toxicity. One convulsed during compression and died of respiratory failure upon decompression. The second developed para-

lysis of the hind legs after compression but survived. The development of congestion and hemorrhage in the heart and lungs frequently seen after exposure to high pressure oxygen was not seen in the shocked animals treated with OHP. The degree of necrosis and congestion seen in sections of intestines, liver, and kidneys of shock dogs who died after OHP therapy was less marked both grossly and microscopically than lesions seen in shock dogs who succumbed breathing room air. However, the degree of oxygen toxicity is a function of time of exposure and degree of compression. A period of exposure of 3 hours at 3 atmospheres is well tolerated, since the changes it produces are rapidly reversible; however, exposures at 3 atmospheres or higher for 4 hours or longer produces irreversible changes, though species variations exist.

SUMMARY AND CONCLUSIONS

Oxygen breathing at 3 atmospheres has been found to check the irreversible trend in hemorrhagic shock and increase the survival rate from 17% in the control shock group to 74%. The beneficial results of OHP are attributed to the increased amount of physically dissolved oxygen which maintain the oxidative processes at the cellular level despite reduced oxygen transport by hemoglobin.

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HYPERBARIC OXYGENATION (OHP) IN MASSIVE PULMONARY EMBOLISM*

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There has been a revival of interest in the treatment of pulmonary embolism since Cooley and Beall[1] reported the first successful pulmonary embolectomy using cardiopulmonary bypass in 1961. Around the same time hyperbaric oxygenation (OHP) was found beneficial in anoxic states, such as carbon monoxide poisoning, peripheral vascular insufficiency and vascular collapse. Since arterial desaturation and tissue anoxia constitue the main sequelae of massive pulmonary embolism, an experimental model was designed to simulate the clinical picture and study the effects of OHP on the biochemical, electrocardiographic changes, and survival rate of such a model. In addition, embolectomy using cardiopulmonary bypass was evaluated as a definitive therapeutic measure for pulmonary embolism.

MATERIAL AND METHODS

Various substances and methods have been used to reproduce pulmonary embolism experimentally. In most instances, foreign material has been used: Barium sulfate, lead phosphate agar, lucite (methyl methacrylate) spheres, Penrose drains, glass beads, starch granules, amniotic fluid, filter paper, cotton fibers, lycopodium spors, seeds,

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wax, autogenous fibrin, etc.[2] Besides, general anesthesia utilizing pentobarbital was used uniformly. We believe these 2 factors modify the experimental model to such a degree that it does not represent any more the clinical picture as it occurs spontaneously. Nash[3] demonstrated a significant fall in cardiac outputs amounting to 44% of control values, in dogs subjected to the usual anesthetic dose of 30 mg./kg. of sodium pentobarbital. In addition, the depressant effect of barbituates on respiration are well known to warrant further discussion. Therefore, we decided to use fresh blood clots and local anesthesia. Thirty-two mongrel dogs with an average weight of 11 kilograms were mildly sedated with morphine sulfate, 1.5 mg./kg. body weight, given 2 hours prior to the injection of the clot. Aseptic technique was observed throughout the procedure. Under local 1% procaine anesthesia, the right carotid artery and external jugular veins were cannulated for monitoring arterial pressure, central venous, or pulmonary artery pressures, and injection of the clot into the right side of the heart. The clot was made by aspirating blood 2.3 cc/kg. body weight and allowing it to stand for 1 hour in a polyethylene tubing 1/2 inch wide. The latter was then connected snugly to a Bardic catheter (No. 16-18) already inserted in the external jugular vein, and the clot injected manually using a 50 cc syringe filled with saline. The electrocardiogram was also monitored during the procedure. Arterial and mixed venous blood samples were drawn before injection of the clot, immediately after injection, and at intervals thereafter depending on the

fate of the animal. The samples were examined for pH, CO_2 content, hematocrit, lactic acid, pyruvate, and oxygen saturation. PCO_2 and plasma bicarbonate were calculated from the Singer and Hastings nomogram.

THE OXYGEN CHAMBER

The animals treated with OHP were cannulated as in the control group, fastened to a wooden board and introduced into the pressure chamber. After injection of the clot, the chamber was pressurized with oxygen to 3 atmospheres absolute within 5 minutes; they were maintained under pressure for about 15 minutes after which they were decompressed gradually. The total time of exposure to OHP was 30 minutes or shorter, depending on the outcome of therapy.

The pressure chamber used in these experiments consisted of a converted autoclave, already described in detail in a previous publication[4].

RESULTS

The control group as well as the high pressure oxygen group consisted of 16 dogs each.

A. Hemodynamic changes. (Fig. 1)

According to the changes in the mean systemic arterial pressure, 4 patterns were discernible:

Pattern I. A moderate to marked rise which occured within 15 seconds of clot injection, remained sustained for a few minutes, and then returned to preelot levels within 5 minutes.

Pattern II. A moderate drop down to 40-60 mm. Hg. within seconds of clot injection, which was not sustained, but returned to baseline

levels within 2 minutes. Pattern III. An instantaneous drop to zero levels which never recovered. Pattern IV. A gradual drop to nonrecordable pressures which remained sustained for about 2-3 minutes followed by a marked rise to hypertensive levels which gradually returned to pre-clot levels.

It was possible to measure the central venous (CVP) or pulmonary pressures (PAP) in the control group, whereas in the OHP group measurements of the CVP or PAP were not possible while the animals were in the chamber. An immediate and marked rise was observed in the CVP, right ventricular pressure and pulmonary artery pressure upon injection of the clot simultaneously with a marked drop in the carotid arterial pressure as illustrated in Fig. 2. In the animals that survived, the pressures were reversed toward their normal values within 2 minutes. In the animals that died the CVP and PAP rose immediately above 75 mm. Hg. while the carotid artery pressure dropped significantly. This trend continued for 3-4 minutes, after which both pressures dropped to zero levels.

B. Electrocardiographic changes.

Most of the animals exhibited sinus bradycardia and arrhythmia prior to clot injection which was attributed to morphine. Upon injection of the clot, sinus tachycardia appeared with frequent premature ventricular systoles. Marked ST depression appeared with inversion of the T wave. Atrial fibrillation was occasionally seen. Terminally, nodal or idioventricular rhythms appeared which ended in ventricular fibrillation or cardiac arrest.

In the control animals that survived the same electrocardiographic changes appeared after injection of the clot; these persisted for about 3-4 minutes, then returned gradually to the preclot pattern.

In the OHP group, sinus tachycardia, ST depression, varying degress of AV block with peaked T wave appeared within seconds after clot injection. However, in 2-4 minutes as the pressure was built up to 3 atmospheres, bradycardia appeared, the ST depression returned to isoelectric levels and the EKG returned to normal patterns in the surviving animals. In one interesting experiment illustrated in Fig. 3, St segment became depressed 20 seconds after the impact of the embolus, and idioventricular rhythm appeared 1 minute later. OHP was started 2 minutes after injection of the embolus. A notched P wave appeared with marked depression of the ST segment. In 4 minutes, a normal electrocardiographic pattern reappeared. The tank ran out of oxygen, however, and the pressure within the tank could not be maintained because of leaks. Within 6 minutes, the T wave became flattened, and in 8 minutes the ST segment became elevated. The dog developed complete atrioventricular block with idioventricular rhythm in 10 minutes.

C. Biochemical changes.

Significant reduction in arterial and mixed venous oxygen saturation associated with a decrease in pH, elevation in lactic acid and a decrease in PCO_2, (though became elevated terminally) were noted in the control group that did not survive. (Figure 6) Similar but less

striking changes were observed in the control group that survived. A tendency towards normal values was observed in the recovery period. (Figure 5)

The biochemical changes observed in the OHP group were remarkable in the absence of arterial and mixed venous oxygen desaturation. The mixed venous blood was bright red and oxygen bubbles developed as soon as the samples were exposed to atmospheric air. The changes in pH, PCO_2, and lactic acid as illustrated in Figure 6 reflected adequate oxygenation of the tissues, in contrast to the anoxic and acidotic changes seen in the control group.

D. Survival rate.

Of the 16 animals comprising the control group, 8 survived giving a rate of 50%. Among the 16 animals treated with OHP, there were 5 fatalities with 11 survivors, giving a rate of 69%. Although the series is too small to make the difference in survival rate statistically significant, further analysis of the data sheds more light on the value of OHP. From a hemodynamic point of view, animals exhibiting Patterns I and II would have survived without any therapy. There were 7 controls and 2 OHP in those groups, all of which survived. Those animals which exhibited marked systemic hypotension rarely recovered. In this study (Patterns III and IV) 9 controls and 14 OHP dogs followed that pattern. There were 9 survivals in the OHP group and 3 in the control group, giving a survival rate of 64% in the OHP group compared to 33% in the control group.

DISCUSSION

Despite a large amount of experimentation much controversy still exists regarding the mechanisms operating after a massive pulmonary embolus. Niden and Aviado[5] using glass beads to produce pulmonary embolism concluded that at least 3 components were responsible for the hemodynamic changes (1) primary mechanical obstruction of the vessels which produces the immediate rise in pulmonary artery pressure, (2) secondary local vasoconstriction, (3) vasoconstriction extending to the other lobes. Williams[6] utilizing the same technique of glass spheres and isolated perfusion of the left lower lobe concluded that the hemodynamic effects were due to mechanical obstruction to the flow of blood through the lungs and were eventually followed by right heart failure. Nelson and Smith[7] found insufficient evidence that neurogenic reflexes play any role in massive embolism. Serotonin[8] (5-hydroxy-tryptamine) has also been implicated in the production of pulmonary hypertension and systemic hypotension, since it produces these same hemodynamic changes when injected intravenously and is present in the blood clot and possibly in the lungs. Parmley, North and Ott[9] stated that the cause of severe but potentially reversible systemic hypotension appeared to be due to a combination of factors including a decrease in cardiac output and reflex changes that influence the systemic arteriolar resistance.

Despite this controversy regarding the pathophysiological mechanisms of massive pulmonary embolism, there is a unanimous

agreement about the resulting peripheral arterial desaturation, anoxemia, and the beneficial effects of oxygen. In 1936, Gibbon and Churchill[10] found that when 86 per cent of the pulmonary vascular bed was occluded, there was a fall in systemic blood pressure and a profound decrease in the oxygen saturation of the arterial blood resulting in death. Inhalation of oxygen increased the oxygen saturation of the blood and maintained life of the animals. According to Binger[11] this arterial desaturation was brought about by the congestion of the pulmonary vessels and the rapid flow of blood resulting in impaired diffusion of oxygen into the blood. In 1939, De Takats[12] et al produced fatal pulmonary emboli by the injection of 2 cc. of an emulsion of potato starch into the central vein of the ear of the rabbit. When oxygen was administered through a tracheal tube, it reduced the dyspnea and protected the whole series from death.

Niden and Aviado[5] considered the anoxemia a potential cause of death and a reasonable direction for therapeutic intervention. The average fall in arterial oxygen content after embolization in 12 dogs amounted to 3 volumes per cent. The fall was often maximal within 5 minutes after embolization, and was considered too soon to be produced by a change in the permeability of the alveolar membrane. Forced ventilation with air did not appreciably alleviate the anoxemia, but administration of 100 per cent oxygen led to prompt and complete removal of the anoxemia. This relief, however, could not be maintained for more than about 30 minutes after which anoxemia reappeared and the animals died despite continuous oxygen administration. From their

perfusion experiment they concluded that pulmonary arteriovenous communications existed and were at least 420 micronin size. Ventilation of the lungs with 100 per cent oxygen decreased the number of these communications while 10 per cent oxygen increased it. Williams[6] noted a 35 per cent fall in oxygen saturation in 31 experiments on dogs breathing room air under constant positive respiration. However, repeated observations of the oxygen saturation on 23 dogs breathing oxygen revealed no significant fall. He did not believe that the unsaturation seen after embolism was due to a form of venous admixture to arterialized blood since a shunt of 50 per cent or greater would have been necessary to cause such a degree of unsaturation. The most likely explanation was the reduction in diffusion capacity of the lung, which improved as new capillaries opened and the area available for gas exchange increased.

Our data corroborate the above conclusions. The hyperbaric oxygen prevents the arterial desaturation and anoxemia of the tissues, not only by increasing the amount of dissolved oxygen in the plasma, but alsobby facilitating oxygen diffusion. This supports the function of the cardiopulmonary system during the sudden hemodynamic changes that follow the impact of the embolus, until compensatory mechanisms are activated. The quick reversal of the hemodynamic, biochemical and electrocardiographic changes following OHP administration as well as the gross and microscopic pathology findings confirm this assumption. It was noted that when the right ventricle and the main pulmonary artery with its 2 major branches were occluded with clots, the chances of

survival were very poor in both the control and OHP groups. The pathologic findings of these animals were correlated with the hemodynamic and EKG findings and it became apparent that the instantaneous cardiovascular collapse following injection of the embolus did not afford the animal an opportunity to compensate and survive. However, in the OHP treated group, the heart maintained its function and was able to displace the clots peripherally into the pulmonary circuit where the capillary surface area is much larger than that of the pulmonary artery.

Although hyperbaric oxygenation can be complicated by oxygen toxicity, the short period of treatment as well as the moderate pressure used avoided such complications. The surviving animals treated with OHP did not exhibit any neurologic sequelae, although 2 dogs became acutely agitated and aggressive immediately following decompression. The development of congestion and atalectasis of the lungs as seen in the histologic sections was attributed to the massive embolus and not to oxygen toxicity since it occurred to the same extent in both groups.

Despite significant improvement obtained experimentally with oxygen therapy of pulmonary embolism, we do not advocate it as the sole means of treatment. We believe that embolectomy using the pump oxygenator remains the definitive treatment. We evaluated this approach in 8 animals and were able to recover most of the emboli. Hyperbaric oxygenation, however, should be considered a supportive therapy in all cases of massive pulmonary embolism. Further experimentation is in progress to evaluate the feasibility of embolectomy following irreversible

pulmonary embolization treated with hyperbaric oxygenation.

SUMMARY

Massive pulmonary embolism was produced in 16 dogs using autologous blood clots. Survival rate in this group was 30 per cent. Sixteen dogs were embolized in a simialr fashion and were treated with oxygen under 3 atmospheres absolute. The survival rate was increased to 69%. The improvement in survival rate was correlated with improvement in the hemodynamic, biochemical and electrocardiographic changes.

ACKNOWLEDGEMENT

We gratefully acknowledge the technical assistance of George Henning, and the help of Doctor Yu Chen Lee in the interpretation of the electrocardiograms.

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LEGEND

Fig. 1 - Graph representing patterns of hemodynamic changes following impact of pulmonary embolus.

Fig. 2 - Graphs representing changes in the electrocardiogram, carotid artery, right ventricular, and central venous pressures before, during, and after embolus, in 2 control animals.

Fig. 3 - Tracings illustrating the effect of OHP on the electrocardiogram (Lead II) of an embolized dog.

Fig. 4 - Graphs showing changes in pH, PCO_2, plasma bicarbonate, oxygen saturation, lactic acid and pyruvate following pulmonary embolus. Group III.

Fig. 5 - Graphs showing changes in pH, oxygen saturation, lactic acid, and pyruvate following pulmonary embolus. Group II.

Fig. 6 - Graphs showing changes in pH, PCO_2, oxygen saturation, lactic acid, and pyruvate following pulmonary embolus and OHP.

APPENDIX E

ALTERATIONS IN BACTERIAL DEFENSE MECHANISM IN THE HUMAN DURING SHOCK

R.M. Ollodart, M.D., A.R. Mansberger, Jr., M.D., R A. Cowley, M.D. and R.W. Buxton, M.D.

Introduction

Assessment of changes in bacterial defense mechanisms in the human associated with shock is of interest from at least two points of view. The clinician, charged with the care of the patient in shock, must use rational prophylaxis and knowledgeable treatment for a not uncommon complication of that condition, infection. Fine[8] has shown reduction in bacterial clearance in animals and Miles and Nevin[17] have demonstrated reduction in local skin resistance to infection with shock. Redfern[22], in a series of 100 consecutive patients with intra-abdominal abscesses noted shock as a complicating factor associated with operation in 59 per cent of the cases, implying increased susceptibility to intraperitoneal contamination. Balch[1], studying soldiers with battle injuries in Korea, noted decreased phagocytosis in casualties with shock. Although most clinicians suspect that there is decreased resistance to infection from shock, documentation of this phenomenon in humans has not been completely accomplished.

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The second point of view relates to alterations in bacterial defense mechanisms which may provide clues as to the role of bacteria and their products in shock. Over a period of years, a group of investigators have collected considerable data which implicate gram negative bacterial endotoxin primarily from symbiotic inhabitants of the gastrointestinal tract[8],[9],[10],[12],[26] Endotoxin is felt by these individuals to be a common denominator at some stage in the various forms of shock. Although there is considerable controversy concerning the role of endotoxin in forms of shock other than septic[16],[24], the impressive body of work accumulated by this group, in animals, excites ones curiosity regarding humans.

Since bacterial clearance is dependent, at least in part, on perfusion of the reticuloendothelial system[8], and local resistance is felt to be partly dependent on perfusion which brings plasma components and white cells to the area[2], it might be argued that change in these particular bacterial defenses result simply from changes of blood flow to the tissues involved. A study of the parameters of bacterial defense which might be impaired even if local blood flow were nearly normal would help clarify changes due specifically to shock rather than simply local decrease in blood flow to an area containing bacteria. For this reason, the parameters studied consisted of the serum or plasma bactericidal titer, the serum complement titer, and the phagocytic index of the peripheral polymorphonuclear

neutrophils. In addition, changes in the serum proteins referable primarily to the complement system were also studied.

Materials and Methods

Bactericidal titer

A suspension of a 16 hour glycerinated trypticase soy agar slant culture of E. coli serotype 0127B8* was made in normal saline. This was washed and adjusted to 10^3 organisms per milliliter, using the Beckman DU spectrophotometer at 650 millimicrons. Stock cultures were kept at 1 - 3°C. One tenth of one milliliter of the suspension was incubated with 0.4 milliliter of serum dilution for one hour at 37°C and then 4.5 milliliter of saline was added to each tube to stop the reaction. One-half milliliter aliquots from each tube were placed in the wells of sterile plastic trays following which 1.9 milliliter of liquid agar was placed in each well. Each serum dilution was tested in duplicate. Sterile Saran Wrap was used to cover and seal the trays which were incubated for 18 hours at 37°C. The number of bacteria in each well was counted as were a series of at least six control wells containing saline instead of serum. Wells containing agar alone controlled contamination. The control wells contained approximately ten bacteria per well. The bactericidal titer was taken as the highest dilution of serum killing 50 per cent or more of the average number of bacteria in the controls.

*Provided by the Department of Microbiology, Walter Reed General Hospital, Washington, D.C.

Complement titer

Hemolytic activity of complement was measured according to a modification of the 100 per cent hemolysis method of Bier and Heidelberger[3]. The 50 per cent method of Mayer was also used in a few cases. The majority of the data reported was in terms of the 100 per cent method which was performed by adding 0.6 milliliter of rabbit anti-sheep cell hemolysin in optimal concentration to 0.2 milliliter of 4 per cent washed sheep red cells and incubating this for 10 minutes at 37°C and read. The highest dilution of serum producing 100 per cent hemolysis times ten equaled the number of 100 per cent hemolytic units per milliliter of undiluted serum. The 50 per cent method was done according to Mayer[15]. Dilutions in the case of the 100 per cent method were done in calcium magnesium saline and in the case of the 50 per cent method triethanolamine buffer pH 7.2 was used.

Phagocytic index

Blood was collected in sterile siliconized tubes with a heparinized syringe. A white count and differential was performed and to 1 cc of blood was added 1 cc of a suitable suspension of E. coli to give 10^3 organisms per polymorphonuclear neutrophil. The mixture was incubated at 37°C with gentle agitation every 10 to 15 minutes and then spun down. The supernatant was discarded and a smear of the cells was stained with Wrights stain. The average number of bacteria in 30 to 50 consecutive polymorphonuclear

neutrophils was determined. Preliminary experiments demonstrated a linear relationship between the number of bacteria per poly and the phagocytic index over a range of 500 to 2,500 bacteria per poly. An easily counted number of around ten bacteria per cell, was obtained in normal blood if 1,000 bacteria per poly were used in the test.

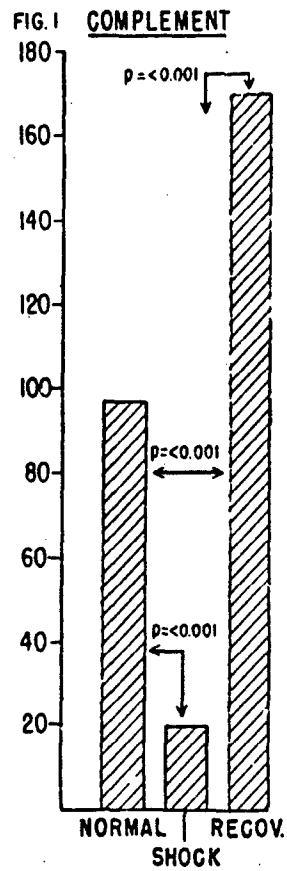
Immunoelectrophoresis

Twenty-five lambda of patients serum was placed in the well of a slide containing barbital buffer, pH 8.4, with an ionic strength of 0.05 in 2 per cent purified agar. This was subjected to electrophoresis in a microelectrophoresis apparatus and then fixed in 2 per cent acetic acid, dried, stained and analyzed in a reflected light scanner. In a similar manner five lambda of serum was electrophoresed and then reacted with 25 lambda of horse anti-human serum placed in wells paralleling the electrophoretic axis. After 24 hours in a humidity chamber, the slides were washed in saline, dried, stained and analyzed.

Blood cultures

Blood was collected aseptically in heparinized syringes and placed in sterile siliconized tubes. Shortly after collection one milliliter of blood was mixed aseptically with liquified trypticase soy agar and poured as a quantitative blood culture in plastic petri dishes. The number of organisms was counted the following day.

Figure 1
Serum Complement in Shock



Figures Represent 100% Hemolytic Units

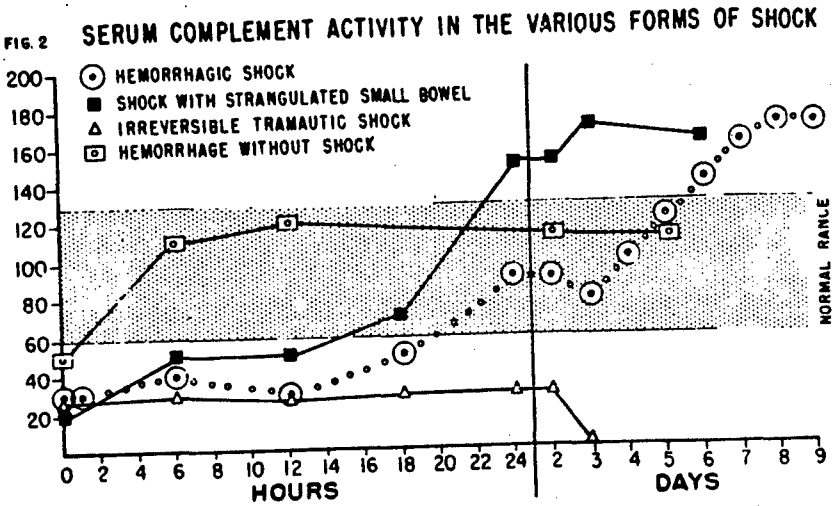
Results

Serum Complement

Figure 1 summarizes results on serum complement tests in patients. Examination of 18 normal subjects by the 100 per cent hemolytic method resulted in a mean titer of 97.3 units, plus and minus 33.8 units (two standard deviations) which agrees with Bier and Heidelberger's values (100 units) for normal humans using this method[3]. Specimens from 16 patients in shock showed a mean value of 20 units, plus and minus 21.8, and the P value for the shock relative to the normal was less than .001, indicating a highly significant decrease in serum complement during shock. In six patients analyzed after recovery from shock the mean value was 170 units, plus and minus 17.8, again with a P value of less that .001 relative to the normal. In six patients undergoing major operation (biliary or gastric) with no active infection or shock, the complement level did not change significantly as a result of the operation.

Figure 2 illustrates the course of typical patients in the various forms of shock. All patients show a low level with shock which remains low for 12 to 18 hours before climbing to above normal levels in those who recovered. Irreversible shock is accompanied by a continuously falling complement titer until death. In two cases of upper gastrointestinal hemorrhage without shock (blood pressure greater than 70 mm.Hg. systolic) the complement

Figure 2



Figures Represent 100% Hemolytic Units

level was depressed to a lesser degree than with shock and returned to normal immediately after transfusion (within 6 hours). This agrees with results of graded bleeding without shock in guinea pigs wherein initially lower complement levels returned to normal within 4 to 5 hours of bleeding[4]. Thus, shock produced a rather specific pattern which differs from operation or hemorrhage alone. This pattern is similar to that produced by infection as can be seen with the influenza patient in Figure 3. Here there is a drop coincidental with the onset of clinical disease and a rebound above normal after recovery.

Since many patients received Dextran (Baxter "Gentran" 6% in N-Saline) and native dextrans are known to be anti-complementary, Dextran had to be ruled out as the cause for some of the low values of complement. "Gentran" incorporated in the test for complement in concentrations ranging from six per cent to .02 per cent had no effect on the hemolytic titer of a series of normal sera. On the other hand, heparin, which is known to be anti-complementary[21], has an inhibitory effect on hemolysis in concentrations of as little as 62 units per cc of serum.

The question of contribution of complement by stored, banked ACD blood has been raised[1]. Table I illustrates the effect of ACD on the hemolytic activity of complement. As can be seen high concentration will inhibit hemolysis probably by tying up divalent cations. In a particular zone of concentration there is a false

Figure 3
Complement, Bacteriacidal Titer
and Phagocytic Index with Influenza

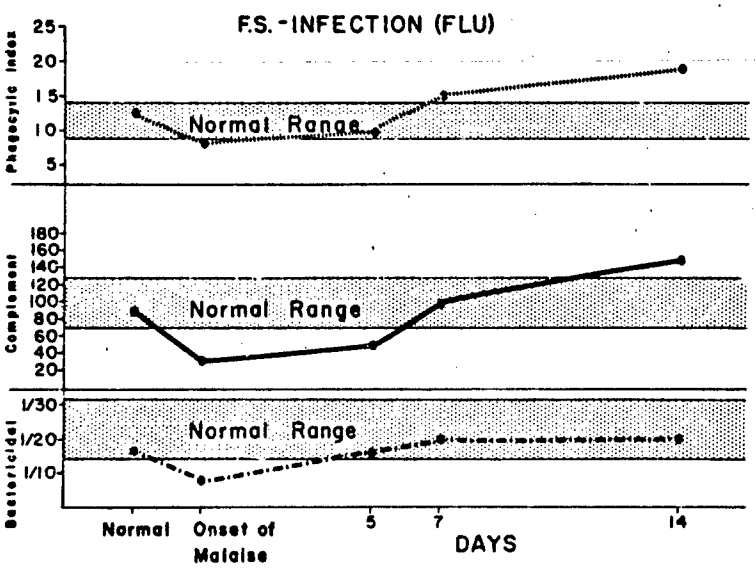


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Table I

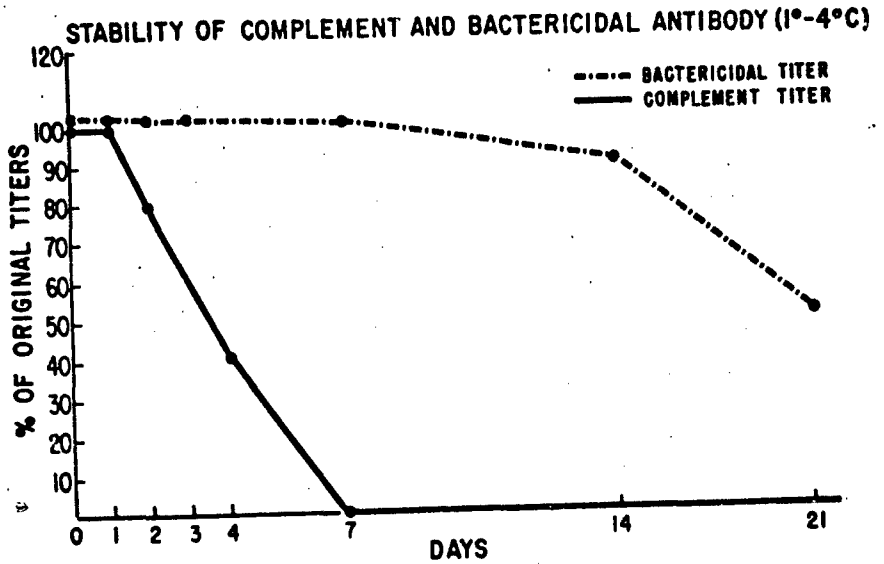
EFFECTS OF ACD ON COMPLEMENT TITRATION

A) Complement titer of serum - 50 units
Titer of ACD 20% - 0 units
Titer of serum + ACD (5%) - 100 units

B) ACD%	Reading	
50	~~1111~~	zone of false low C^1 titer
25	~~1111~~	
12.5	~~111~~	
6	~~111~~	
3	-	zone of false high C^1 titer
1.5	+	
.75	~~11~~	
.35	~~11~~	
Control	~~11~~	

Note: Control tubes contain sufficient complement to just give partial hemolysis designated as (++). No hemolysis = (++++) and complete hemolysis = (-).

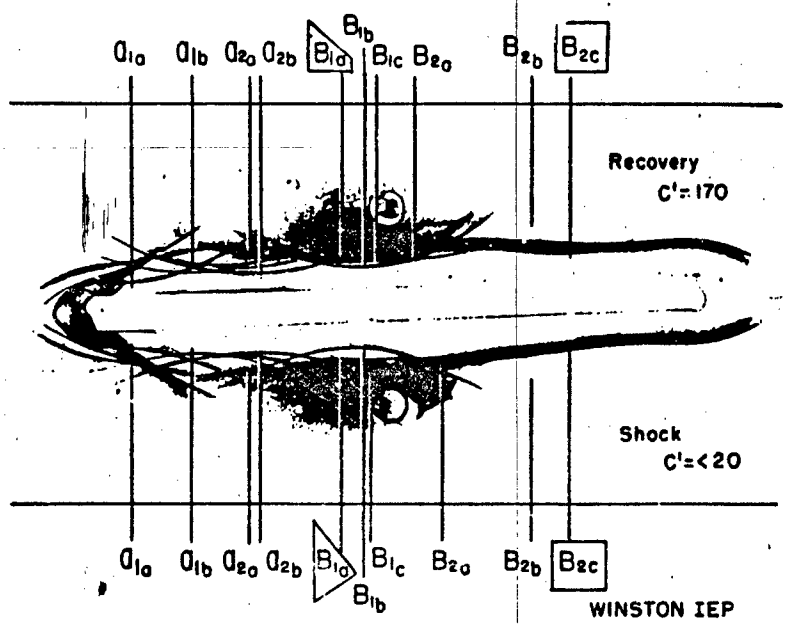
Figure 4


STABILITY OF COMPLEMENT AND BACTERICIDAL ANTIBODY (1°-4°C)
BACTERICIDAL TITER
COMPLEMENT TITER
% OF ORIGINAL TITERS
120
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
7
14
21
DAYS

positive effect on the hemolysis test which can give an impression of a high titer of complement whereby a serum which has a titer of 50 units showed 100 units with 5 per cent ACD. Hemolytic activity, therefore, cannot be estimated in stored bank blood with ACD solution. Figure 4 illustrates the rate of deterioration of hemolytic activity of complement in serum stored at 1 - 4°C. One would expect the complement in ACD stored bank blood to deteriorate at a similar rate unless there were some very unusual preservative effect present. It appears, therefore, that stored blood will contribute little if any active complementary activity to the recipient of transfused bank blood.

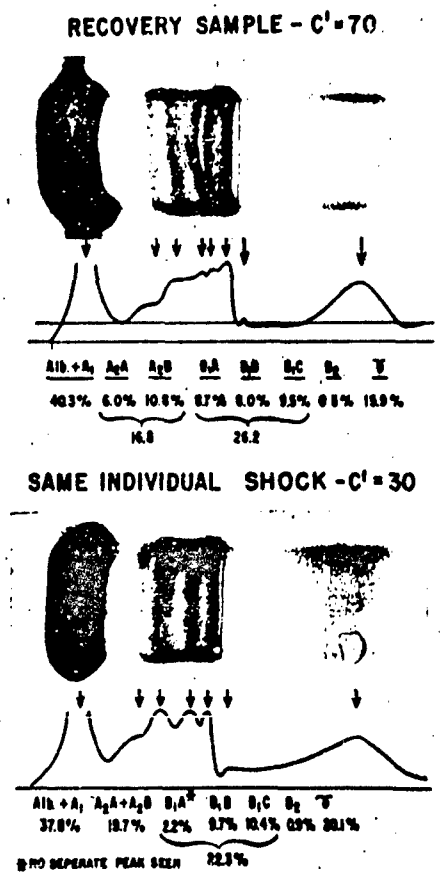
Electrophoresis and immunoelectrophoresis of serum from patients in shock was compared to the other parameters such as complement activity. Comparison of immunoelectrophoresis of sera from patients in shock and during their recovery showed a consistent difference in virtually all patients (Figure 5). There was a decrease in a particular line associated with shock. This was a rapid Beta 1 globulin which appeared in large quantity in recovery samples. This line corresponded to a dark peak seen in electrophoresis between the Alpha 2 and Beta 1 globulins and designated as Beta 1A (Figure 6). Figure 7 illustrates the fact that this peak varied in close correlation to serum complement activity as did the Beta 1A line on the immunoelectrophoresis in shock patients.

Figure 5
Immunoelectrophoretic Pattern
In Shock and Recovery



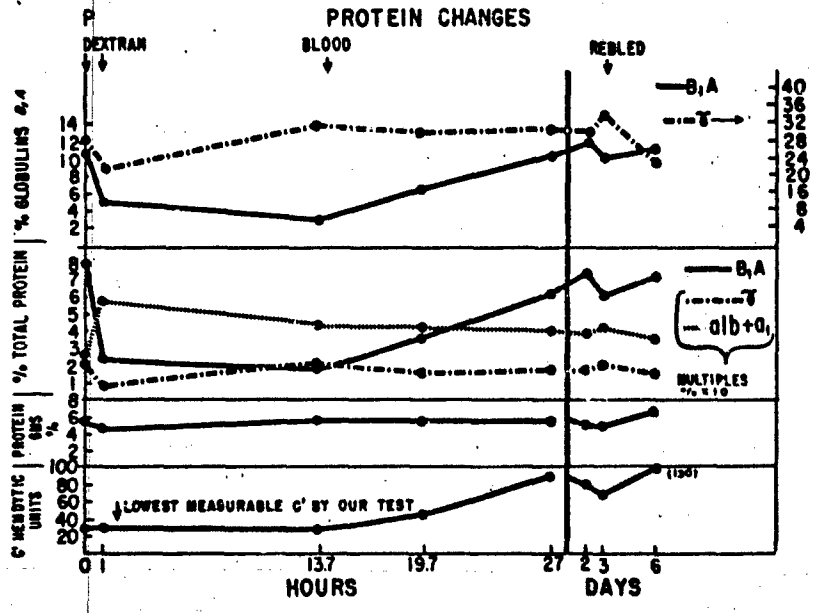
Note: B_1A is vague in shock and is pronounced in recovery.

Figure 6
Electrophoretic Pattern in
Shock and During Recovery



181

Figure 7



Note: There is a correlation in shock and recovery between hemolytic complement and B_1A.

Figure 8
Immunoelectrophoretic Comparison
of B_1 Globulins in Fresh and
Incubated Serum

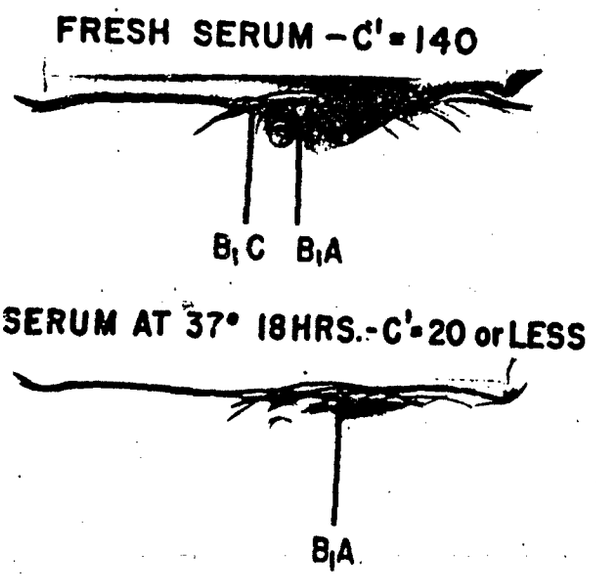
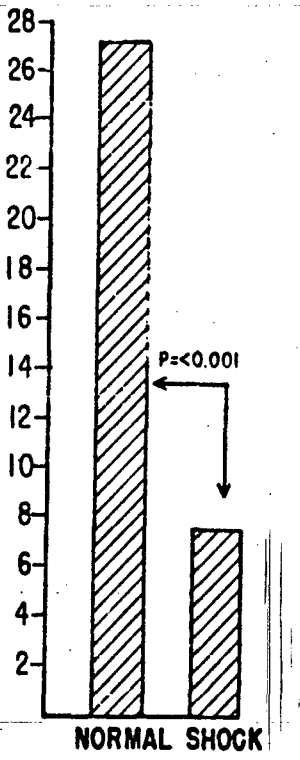


FIG. 9

BACTERIC. TITER



Reports in the literature indicated that this serum protein might correspond to the inactivated form of the first component of complement[18]. Accordingly, fresh serum from a patient who recovered from shock and had a complement titer of 140 units was compared immunoelectrophoretically with the same serum after incubation at 37°C for 24 hours resulting in an inactivation of complement. Comparison (Figure 8) revealed the presence of a slow moving Beta 1 globulin (Beta 1C) in fresh serum with a faint Beta 1A line. After incubation when complement activity was minimal, the Beta 1C was gone and a heavy Beta 1A was present. This Beta 1A corresponded to the first component of complement[18], and to the protein noted to fluctuate in parallel with the complementary activity in shock patients. It was concluded that it was, in fact, the first component complement in an inactivated form and this form was seen because electrophoretic analyses were performed days after sera were drawn or after freezing and thawing thus inactivating the first component of complement. The possibility that the inactive form may appear in the plasma of the patients as a result of shock per se was not ruled out.

Bactericidal titer

The bactericidal titer against E. coli was not assayed when patients were receiving broad spectrum antibiotics. Figure 9 shows a summary of the results on the bactericidal tests. In 15 normal

Figure 10
Complement, Bacteriacidal Antibody, and
Phagocytic Index in Shock and
Major Surgery

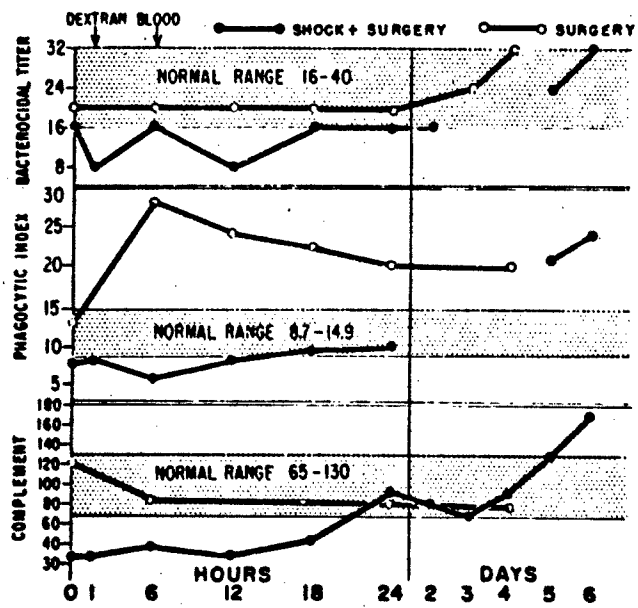


Table II

BACTERICIDAL TEST-EFFECTS OF HEATING AT 56°C FOR 30' AND OF SELECTIVE ABSORPTION

Serum Dilution	Normal Serum		Heated Serum		E. coli Abs.Serum	
Undil.	0	0	6	5		
1:2	0	0	7	10	6	5
1:4	0	0	8	9	9	6
1:8	0	1	11	9	11	11
1:16	2	2	12	9	10	10
1:32	6	10	8	14		
1:64	10	12	7	10		
1:128	10	8	10	7		
saline control	7	10	10	12	7	8

*E. coli absorbed serum left with complement titer of 90%

Average = 9
90% = 4 or less

Figures in box represent the number of bacterial colonies.
Titer of untreated serum = 16.

subjects the mean titer was 27.2, plus and minus 11.8, while 11 patients studied during shock showed a mean of 7.6, plus and minus 9.2, (P value of less than .001). No rebound was noted in association with recovery from shock. The titer returned to normal more promptly after resuscitation than did complement titers. Figure 10 illustrates the typical course of the bactericidal titer in hemorrhagic shock. A series of six surgical patients showed no significant alteration of the bactericidal titer during or after operations.

The measurement of the bactericidal titer is thought to involve at least two parameters, complement and "antibody," which seem to cooperate in this process[20],[13]. In the case of some strains of E. coli it has been noted that complement may play little or no role, at least in terms of subsequent loss of its hemolytic activity[21]. This seemed to be the case for the E. coli serotype 0127B8. Table II shows the effect of heating and adsorption on the bactericidal titer. Heating, which destroys complement and natural antibody[20], removed completely bactericidal effect. If the serum is adsorbed with E. coli which has been heat killed at 60°C. for one hour, a ratio of coli organisms to serum can be found wherein 90 per cent of the complementary activity can be left while virtually all the bactericidal power is removed. Thus, the combination of so-called natural antibody and heat killed E. coli

Figure 11

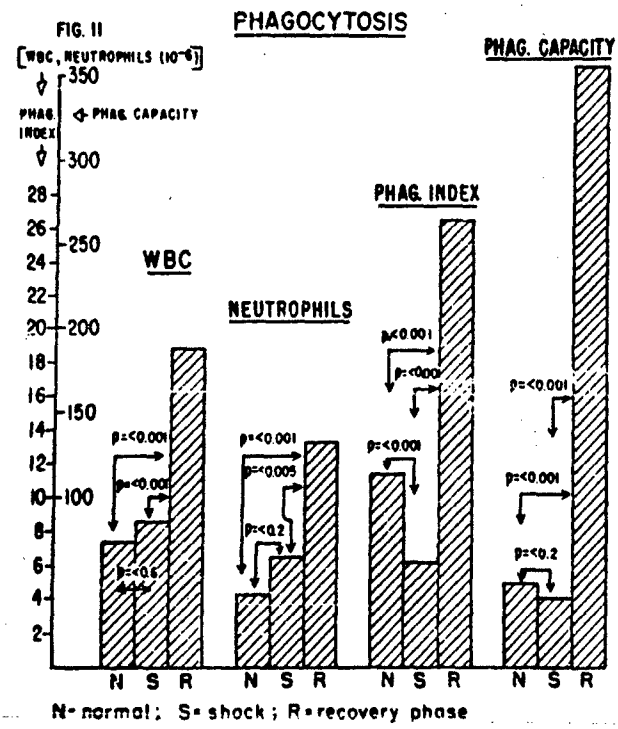


FIG. II
PHAGOCYTOSIS
[WBC, NEUTROPHILS (10⁻⁶)]
PHAG. INDEX
PHAG. CAPACITY
350
300
250
200
150
100
28
26
24
22
20
18
16
14
12
10
8
6
4
2
WBC
NEUTROPHILS
PHAG. INDEX
PHAG. CAPACITY
p=<0.001
p=<0.005
p=<0.2
p=<0.6
N S R N S R N S R N S R
N= normal; S= shock; R= recovery phase

does not result in inactivation of complement. Further evidence that the bactericidal titer as we have measured it is relatively independent of complement is provided as shown by Figure 4 where the rate of deterioration of complement activity is compared to that of bactericidal effect in serum stored aseptically at 1 to 4°C. Complement activity shows a sharp drop after 24 hours and is virtually gone by one week. Bactericidal effect remains stable until about two weeks after which it very slowly drops so that at three weeks it is still at 50 per cent of its original value.

Phagocytic index

The white blood cell count, differential, phagocytic index and phagocytic capacity were measured during and after shock. Phagocytic index was defined as the average number of bacteria per polymorphonuclear neutrophil after incubation with E. coli as described in Materials and Methods. Phagocytic capacity was defined as the number of bacteria that could be phagocytized by the neutrophils in one milliliter of whole blood and was obtained by multiplying the phagocytic index by the number of neutrophils per cc. of blood. Figure 11 summarizes the results of these studies. The phagocytic index was significantly depressed whereas the phagocytic capacity was depressed to a degree which was not statistically significant during shock. The reason for this appeared to be an overall increase in the number of neutrophils

Table III

EFFECTS OF HEPARINIZED PLASMA COMPONENTS ON PHAGOCYTOSIS

	Hemolytic C^1 Titer	Bactericidal Titer	Phagocytic Index
A)			
1)whole blood (heparinized)	0	20	19.5
2)plasma heated 56° 30'	0	0	10.8 (55.3%)
3)plasma replaced with saline			10.3 (52.8%)
4)plasma absorbed with E. coli in ratio leaving 90% of complement activity in serum	0	0	10.0 (51.2%)
B)			
1)whole blood		16	22.4
2)plasma replaced with saline			12.1(54%)
3)plasma of 1 week old ACD blood		8(50%)	11.0(49.1%)
4)plasma of 6 week old ACD blood		12(75%)	15.2(67.8%)

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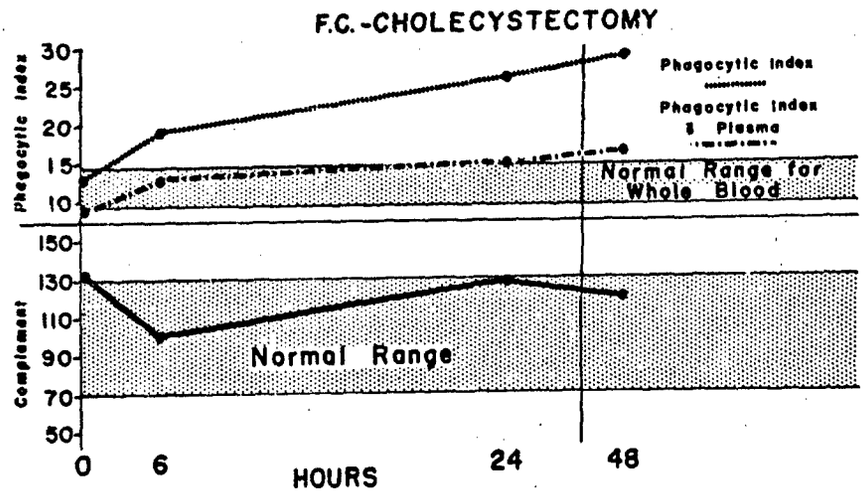
during shock, possibly associated with hemoconcentration. One can conclude that the ability of individual white cells to phagocytize bacteria is decreased in shock but this is partially compensated for in the blood stream by hemoconcentration. Figure 10 illustrates the course of the phagocytic index during shock and after major operations compared to bactericidal titer and complement. The correlation with complement in shock was consistently close and could be explained by the opsonizing action of its various components. In postoperative surgical patients there was a consistent statistically significant postoperative rise in phagocytosis with no significant change in complement or bactericidal titer. Since this rise seemed to occur independent of measured plasma factors, experiments to test this situation were performed. Table III A illustrates such an experiment wherein plasma was removed and either heated at 56°C for 30 minutes or absorbed with killed E. coli and then replaced or replaced with saline. The heating of the plasma which is sufficient to destroy natural antibody and complement will reduce the phagocytic index by 40 to 50 per cent. The same occurs when the plasma is adsorbed with heat killed E. coli in such a concentration that will remove the bactericidal effect from serum but still leave the complement hemolytic activity present. Therefore, approximately one-half of the phagocytic activities of whole blood are associated with the plasma components and one-half with properties of the white cells themselves. Table III B illustrates the fact

Figure 12
Phagocytic Index with and
without Plasma Factors in
Major Surgical Cases



that replacement of the plasma with plasma of stored ACD blood from one week of age to three weeks of age results in reduction of phagocytic index which is roughly proportional to reduction of bactericidal effect, a reflection of opsonizing effect of natural antibody. Since the surgical patients who showed a postoperative rise in phagocytosis, did so without corresponding rise in bactericidal antibody or complement, we wondered if this were due to a change in the white cell itself initiated by the stress of operation. An experiment to test this was performed wherein phagocytic index was measured in a surgical patient both in terms of the whole blood and in terms of the blood with the plasma factors removed. As can be seen in Figure 12 there was a rise in the phagocytic index when the plasma was removed, which was parallel to the rise which occurred in the whole blood. These observations suggest that the rise in phagocytic index occurring after operation is a result of direct stimulation of the white blood cell itself whereas the rise in patients who are recovering from shock seems to be at least in part a reflection of an increase in plasma opsonizing factors such as complement and bactericidal antibody.

Blood cultures

Blood cultures were monitored in all patients with each sample drawn. The only positive cultures were obtained in three patients with septic shock and one patient who had traumatic shock and a terminal pneumonia with septicemia. The changes noted in the other

parameters could not be ascribed to blood stream invasion by bacteria except in the case of septic shock although the patterns resemble those produced by infection (Figure 3).

Discussion

Examination of bacterial defense mechanisms in the human during shock was undertaken in order to shed light on the relationship of septic complications to shock as well as to investigate certain theories concerning irreversibility[8]. Patterns in complement titer showed a consistent decrease during shock which persisted for 8 to 12 hours after resuscitation and then rebounded above normal during recovery. Phagocytic indices followed a similar pattern which was well correlated with complement titer and probably reflect opsonizing effect of complement at least in part[7]. Bactericidal titer seemed to be independent of complement hemolytic activity but was also depressed during shock, coming back to normal range soon after resuscitation. Evidence presented rules out direct effects of Dextran or ACD blood as causes of these changes except for their effect on promoting perfusion of vital organs and recovery. Stored banked blood may contribute some bactericidal antibody, but it is unlikely that complement effect or viable neutrophils are contributed for phagocytosis. The stress of a surgical procedure alone cannot duplicate these patterns except for a rebound in phagocytosis which seems to be mediated almost entirely through the white cell itself. Hemorrhage without shock produces a pattern of

moderate depression of complement with immediate recovery after resuscitation and no rebound. Thus, the shock pattern is unique and corresponds to one of infection with depression while the offending organism is present in the blood stream and is absorbing complement and bactericidal antibody, and a rebound after it is eliminated. Except in patients with septic shock, no bacterial invasion of the systemic blood could be demonstrated to account for these patterns. Portal blood was not investigated nor was the possibility of a viral organism as a cause for this ruled out.

A similar pattern to that occurring in shock is produced in mice by injection of killed E. coli cell walls[23]. Similar patterns have been observed in rabbits[11],[24],[19] and mice[25] following injection of endotoxin. Absorption of certain other polysaccharides such as zymosan would presumably be capable of producing a similar pattern[20]. During the depressed phase of shock in mice Rowley[23] and Schaedler and Dubos[25] found that their animals were very susceptible to infection, and during the rebound phase found them relatively resistant. It is suggested that the same may be true for humans so that an <u>increased susceptibility to infection occurs with shock</u>. Graded hemorrhage without shock in the guinea pig has resulted in depression of complement levels and restoration to normal within 2 to 3 hours[4]. No rebound was noted. This is compatible with the pattern shown in this presentation for hemorrhage without shock in the human.

The similarity of the shock pattern to that produced by endotoxin leads one to speculate on the absorption of antigenic material possibly related to E. coli, in the human during shock as a cause for the patterns[14]. Direct evidence for the presence of such material has been hampered by the lack of a good in-vitro test for such a substance. In-vivo neutralization of endotoxin like material by natural antibody and complement may hamper its immunological identification.

Summary and Conclusions

1. Complement hemolytic activity, bactericidal effect of serum, and phagocytic index all show a depressed phase during shock in the human.

2. After recovery from shock complement and phagocytic index show a rebound phase well above normal values. Such a rebound has not been found for bactericidal effect.

3. Hemolytic activity of complement cannot be accurately measured in ACD blood plasma because of the effects of ACD on this test. ACD plasma does not seem to contribute significant opsonizing complement. It would contribute only minimal amounts of bactericidal antibody, and virtually no viable neutrophils. Except for contributing to the resuscitation of a patient and the perfusion of vital organs which produce the factors that have been studied, banked blood has little direct effect on the bacterial defense mechanisms measured in this study.

4. Shock in the human results in an increase susceptibility to infection during the period of shock. After recovery there is a rebound reaction with presumed increase resistance although direct evidence for this is lacking in the human.

5. The patterns observed in shock patients were not due to the nonspecific stress of operation nor were they produced by hemorrhage alone but were unique for shock infection or the absorption of antigenic material such as endotoxin or related polysaccharides.

6. Surgical cases uncomplicated by shock or infection show a rise in phagocytosis which is unaccompanied by a rise in complement or bactericidal antibody following operation. There seems to be a direct effect upon the white cell produced by the stress of the surgical procedure.

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APPENDIX F

HEMORRHAGIC SHOCK IN DOGS TREATED WITH ISMELIN

A STUDY OF SURVIVAL RATE

Peripheral resistance is a major factor in hypertension as well as in hemorrhagic shock. Since guanidine sulfate is used to control hypertension, affects vasal dilatation and therefore better oxygenation of tissue, it was thought to be of value in the treatment of irreversible hemorrhagic shock. Pre-treatment value was particularly investigated since the action of guanidine sulfate as a potent antagonist to pressor responses has been demonstrated to be effective from 4 days to 3 weeks, following a single I.V. injection (Maxwell et al).

Irreversible hemorrhagic shock was induced in 70 dogs using a modification of the Fine technique. Blood pressure was kept at 30 mm/Hg over a period of 2-1/2 hours. Thirty-five dogs were used as controls; the remaining 35 were treated with guanidine sulfate in the following manner:

Group I - 5 dogs. guanidine sulfate 10 mg/kg in approximately 100 cc saline - slow I.V. drip - administered during the shock period. Survival rate was 0%.

Groups II, III, IV and V - 5 dogs each - were pre-treated with 10 mg/kg guanidine sulfate given I.V. and administered at various rates (2- 60 min.).

Group II - 24 hr. pretreated - survival rate - 40%

Group III- 40 hr. pretreated - survival rate - 80%

Group IV- 72 hr. pretreated - survival rate - 80%

Group V - 1 week pretreated - survival rate - 100%

Group VI- 10 days pretreated- survival rate - 100%

Group VII- 2 weeks pretreated - survival rate - 100%

In the control group - survival rate - 17.1%

In groups VI and VII, the dogs femoral artery was cannulated before guanidine sulfate was administered in order to observe the immediate effect of the drug on the blood pressure.

It was found that when guanidine sulfate was administered by syringe and undiluted over a 2-3 minute period, the blood pressure would drop sharply (28 mm/Hg average) within 1-1/2 minutes from the start of the injection, then suddenly overcompensate, reaching a peak (35 mm/Hg above base line average) in about 7 minutes. Blood pressures then returned to normal at an average time of 26 minutes and remained at base line level + 5 mm/Hg. as long as the animals were kept on the manometer (3-6 hr.).

However, when guanidine sulfate was diluted in approximately 100 cc of normal Saline and administered in a slow I.V. drip of 30 - 60 minutes duration, the fluctuation in blood pressure described above was reduced to less than one-fourth in magnitude.

On the day shock was induced, the baseline pressure in all the animals was found to be an average of 12 mm/Hg. lower than it had been before guanidine sulfate had been given ten and fourteen days earlier. Average bleed-out volume was 53 cc/kg. Average time of hemorrhage necessary for blood pressure to drop to 30 mm/Hg. was 10.7 minutes.

Convulsions, extreme rigidity of skeletal muscles and respiratory difficulties, which were observed in almost all of the control animals were found to be much less severe and, in most cases, totally absent in the dogs treated with quanidine sulfate.

The animals in groups V, VI, and VII tolerated shock particularly well and were observed to be alert and without apparent ill effects by evening of the same day shock was induced.

CONTINUATION STUDIES ON d-ALDOSTERONE IN NORMO TENSIVE AND HEMORRHAGIC SHOCKED DOGS

The following study was undertaken in order to determine major effects of d-aldosterone on electrolyte balance, metabolism and blood gases in dogs subjected to hemorrhagic shock, as well as in the normotensive dog. Shock was induced by using a modification of the Fine technique.

Healthy mongrel male dogs were used in this survey. The animals were divided into three groups of ten dogs each.

Group I: was subjected to 2-1/2 hours of hemorrhagic shock at 30 mm/Hg. and treated with tritated d-aldosterone (1.6622 u curies/ml.)

Group II: Same procedure as Group I, except no d-aldosterone was given.

Group III; Was not subjected to shock, but d-aldosterone was administered at the same time and rate as Group I.

All dogs were pre-treated with morphine sulfate (1/8 gr./kg) and received a slow I.V. drip of normal saline (10 cc/kg) during the shock period, including the normotensive group (III).

In the treated shock (I) and treated normotensive group (III), d-aldosterone (.05 mgm/kg) was added to the saline drip 30 minutes after blood pressure stabilized at 30 mm/Hg in the shock animals (I). All dogs had their urinary bladders catheterized. Arterial and venous blood samples as well as urine specimens were obtained at the following times:

1) baseline

2) shock (15 min. after stabilization at 30 mm/Hg of shock groups I and II

3) pre-reinfusion (of shock groups I and II)

4) 30 min. post-reinfusion of groups I and II

5) pre-death or pre-sacrifice

Serum Chlorides: No significant changes could be observed between the three groups.

Serum Potassium: It was found that d-aldosterone adds the retention of potassium within the cell. The rise of potassium levels was found to be slightly depressed in the treated shock group (I), as compared with the untreated shock group (II).

Serum Sodium: It appears that the administration of d-aldosterone greatly enhances the excretion of sodium into the urine. Both treated groups I and III showed a decrease in serum sodium levels (in spite of the infusion of normal saline) during and after d-aldosterone administration, as compared with the untreated group II. In normotensive group III serum sodium levels were well below baseline at the time of the last sample.

Serum Ammonia: No significant changes could be observed between the three groups.

Serum Glucose: No significant difference between the treated (I) and untreated (II) could be demonstrated.

Glucose levels in both groups rose sharply, reaching their peak shortly prior to reinfusion (Sample #3), then underwent a drop which was sustained through

the last sample and went below baseline values. In the normotensive group (III), a slight initial drop of serum glucose was noted during d-aldosterone infusion, also reversing itself at the time of sample #3 and returning to baseline before final samples were taken.

Lactates: No significant difference could be observed between the three groups.

Pyruvates: No significant effect on d-aldosterone on pyruvates could be demonstrated.

Plasma Proteins: d-aldosterone appears to have little, if any, effect on plasma, protein levels in the treated shock group I, as compared with the untreated shock group II. Normotensive group III shows a slight but consistent drop of plasma protein levels throughout the d-aldosterone infusion.

Arterial pH: During the early stages of the experiment, both the treated shock and normotensive groups (I and III) tended to rise slightly. The untreated shock group (II) suggested a drop of pH during the same time period.

Venous pH: No significant difference between the treated (I) and untreated (II) shock groups could be noted. In both groups values dropped initially and then rose during the later stages of the experiment after sample #3. Values in group III remained much the same throughout the experiment.

Arterial Hematocrit: No significant difference could be observed in shock groups I and II.

Venous Hematocrit: The same pattern as with the arterial hematocrit was found.

Arterial Oxygen Saturation: Little difference could be observed between the three groups. d-aldosterone does not seem to have any appreciable effect on arterial-oxygen-saturation in shock.

Venous Oxygen Saturation: Was slightly improved by d-aldosterone. In the normotensive group (III) values tended to rise above baseline level during d-aldosterone administration and returned to near baseline sometime after the end of infusion.

Arterial P_{CO_2}: No significant difference in arterial P_{CO_2} levels could be observed between the treated and untreated shock groups (I and II).

Venous P_{CO_2}: No significant difference between the treated (I) and untreated (II) shock groups could be observed.

Urinary Sodium: Excretion of urinary sodium showed a striking rise in both the treated shock (I) and treated normotensive (III) groups from 25 meq/l to 94 meq/l, at the time of the previous study. Since this rise, however, was mainly attributed to the infusion of Saline - this determination was eliminated in the present experiment.

Urinary Chloride: During the initial stages of shock, d-aldosterone seems to depress the excretion of chloride into the urine.

Urinary Potassium: As would be expected, potassium excretion is retarded by d-aldosterone. This was particularly noted in the treated shock group (I) and slightly less pronounced in the normotensive group (III).

Urinary Proteins: No significant difference could be observed between the three groups.

HEMORRHAGIC SHOCK IN DOGS TREATED IMMEDIATELY
PRE AND POST RE-INFUSION OF BLOOD WITH ALDOSTERONE

ALDOSTERONE

A) After 2 1/2 hours of hemorrhagic shock - post re-infusion.

B) After 2 hours of hemorrhagic shock.

Hemorrhagic shock was induced in 20 dogs using our standard modified Fine technique.

Group A. In 10 dogs blood pressure was kept at 30 mm/Hg over a period of 2 1/2 hours. The dogs were then re-infused with their own blood. After completion of re-infusion, .1 mg/kg aldosterone was administered i.v. in 10 cc/kg of normal saline.

Group B In this group of 10 dogs aldosterone was given (same dose and method as in Group A.) after 2 hours of blood pressure at 30 mm/Hg. After an additional 30 minutes of hypovolemia the shed blood was re-infused.

Average Volume of Blood shed:

Group A: 51.7 cc/kg

Group B: 50.7 cc/kg. - However, an additional bleeding of average 6.6 cc/kg was necessary in this group to hold blood pressure at 30 mm/Hg during and after the aldosterone infusion.

Results:

Group A): Out of the ten dogs, seven died within 24 hours post-shock.

In all of the dogs which succumbed, bloody diarrhea was observed either during the latter part of the shock or re-infusion period. Autopsy on these animals primarily revealed evidence of severe hemorrhagic enteritis. The other three animals survived indefinitely without apparent ill effects.

SURVIVAL RATE - Group A 30%

Group B): In this group two out of the ten dogs died in less than 24 hours post shock. The other eight animals survived indefinitely. Bloody diarrhea was observed in two of the survivors as well as in both of the dogs which died. From autopsy of these two animals revealed the same findings as were seen in Group A.

SURVIVAL RATE - Group B 80%

The control group (35 dogs) which had been done previously had a survival rate of 17.1%.

Average time of initial hemorrhage to reduce blood pressure to 30 mm/Hg.

Group A) 7.5 min.

Group B) 7.1 min.

APPENDIX G

STUDY OF THE EFFECTS OF NEOMYCIN PRETREATMENT ON DOGS SUBJECTED TO ACUTE CORONARY OCCLUSION

EXPERIMENTAL OBJECTIVE

Previous studies with dogs subjected to massive coronary occlusion and resulting infarction with antibiotic pretreatment have indicated an increased survival rate. The basis for such a relationship is derived from the hypothesis of Fine who has theorized that one of the basic causes of irreversibility in shock is endotoxins of bacterial origin.

The most obvious source of such substance would be from the natural flora of the bowel which must enter the blood stream due to a change in permeability of the bowel brought about by a biologically disruptive event. Coronary occlusion and infarction could bring about such an abnormal migration of intestinal bacterium.

If the gut were sterilized by an administered antibiotic, then there would be a decrease in the endotoxins, causing irreversible shock after the occlusion in the animal survived the initial cardiac insult. For this reason, the cause of death must be divided into two categories; that of death due to immediate cardiac insult which would occur within minutes of the time of ligation and death due to shock resulting from the occlusion which would occur later because of the events necessary to bring about the condition of irreversible shock. The pretreatment with an antibiotic should then bring about a decrease in these delayed deaths.

To test this hypothesis, an experimental preparation of occlusion by ligation to a series of dogs pretreated with Neomycin was undertaken.

MATERIALS AND METHODS

In this series, the left circumflex artery of 33 dogs was occluded by a single ligation of 3-0 surgical silk. The ligation was executed by means of a two-to-three inch anterior lateral incision through the fourth intercostal space on the left side of the chest. The dogs were anesthetized with approximately 25 mg/kg of Na Nembutal. During the operative procedure, the ventilation of the lungs was maintained by a Harvard respirator at a rate of sixteen strokes per minute and at a volume of approximately 400 cc per stroke. As a means of demonstrating infarction, preligation, postligation, and daily electrocardiograms were taken on the standard limb and V leads. Electrographic changes were monitored on lead #2 for thirty minutes following the ligation.

The thirty-three dogs were divided into two groups: One group of twenty-three dogs were pretreated with a daily dose of 0.5 gm. of Neomycin administered twice a day for three days preceding the operative procedure. The other fifteen dogs comprised the control group which received no operative pretreatment. Fibrinogen levels and clotting times were performed on five of the pretreated and three of the control dogs to obtain an indication as to whether there was an alteration of the coagulating properties of the blood as a result of partial sterilization of the gut. The extent of sterilization was determined by culturing fecal material on a sterile applicator stick which was inserted into the dog's rectum immediately before operation.

All survivors were sacrificed between five and eight days after the

operation with a lethal dose of Na Nembutal. The heart was removed and the exact location of the ligature was determined in terms of its distance from the left common coronary artery. Topical measurements were taken to determine the area of perfusion by the ligated vessel. An angiogram was taken with contrast media injected into the circumflex artery proximal to the ligation to verify complete occlusion by the ligation. The ventricular myocardial muscle of each heart was then cut into four sagital sections in order to obtain a visual observation of the extent of the infarct. The heart was then fixed with 10% Formalin.

	IMMEDIATE DEATH	DELAYED DEATH	SURVIVAL	TOTAL
PRETREATED	7	0	13	20
PERCENT	35%	0	65%	
CONTROL	5	5	5	15
PERCENT	33.3%	33.3%	33.3%	

A total of 35 dogs surgically ligated, of which there was a total of 18 survivals. On a percentage basis, twice the number of pretreated dogs survived as compared to the control dogs (Table I).

In the immediate death category of the non-surviving group, the distribution was equal. All dogs died of ventricular fibrillation. There were no delayed deaths in the pretreated dogs, whereas thirty-three percent of control dogs died 1 - 24 hours after ligation.

From the data presented in our study, there is no significant change in the death rate due to immediate cardiac trauma as a result of pretreatment of Neomycin sulfate. However, the incidence of delayed deaths appears

to be significantly decreased while the survival rate is increased as compared to the control dogs.

All bacterial studies after pretreated with Neomycin were essentially negative.

The incidence and degree of infarction was established on the basis of electrocardiograms, and gross examination of the excised heart. All the dogs, except one control, showed a typical pattern of infarctions as evidenced by T wave and ST segment changes, and resultant pattern of scarring as indicated by Q wave changes. The anterial and lateral portion of the left ventricular wall on examination were yellowish in color and solid in consistency with the most extensive changes in the papillary muscle. The extent of these changes varied in dog to dog, but however the occurrance of infarction could be questioned in only one dog in the controlled group. The dog showed only slight changes in electrocardiogram and on gross examination there appeared no evidence of infarction. Angiograms and gross examination indicated that ligations were completed and intact.

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